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Original Articles

"Gynaecological cancer screening" or "cervical screening?" The case of Hungary

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Summary

In Hungary, "gynaecological cancer screening" by gynaecologists having interest in oncology has had a long history. The screening tool was colposcopy alone embedded in complete gynaecological examination. Later on smear-taking for cytology has been added. This screening protocol has survived until now both in the gynaecological community and public. In the meantime, as it proved its effectiveness, cytological examination has been internationally recommended,\ as sole method of organized cervical screening; in case of non-negative test result, gynaecological examination including colposcopy is justified. Smear-taking can be undertaken by trained paramedical personnel. The authors have made an attempt to argue the use of "cervical screening" instead of "gynaecological cancer screening", which is deeply entrenched into both professional and public consciousness in Hungary.

Key words: Cervical screening; Colposcopy; Cytology; Health visitors.

Introduction

Hungary is carrying a heavy burden of cervical cancer. Regarding mortality, it may be found in the list of mortalities in the last quarter among the European countries (Figure 1). Cervical screening, as a public health policy, has proved its effectiveness in terms of reduction of morbidity, and mortality from the target disease in the target population (Figure 2) [1], and a wide application of morphology-based cervical cytology (Pap smear) as screening tool is strongly recommended by international bodies [2, 3]. Hungary (a Central-Eastern European country, population ten million, one of the "countries in transition" which joined the European Union in 2004), cervical screening had undergone several developmental stages [4].

History of cervical screening on Hungary

In Hungary, the history of opportunistic cervical screening dates back to the late 1950s. At the beginning, the screening tool was *colposcopy alone,* sporadically applied by gynaecologists engaged in practicing oncological gynaecology. In 1954, a ministerial decree was issued, declaring that "mass screening must be conducted in such a way that each woman over 30 years of age be screened by colposcopy" (MOH 8834/31/1954). There are no data on its effect.

In the meantime, *exfoliative cytology* has been extensively investigated, as a method of early detection of potentially pre-cancerous lesions (cervical intraepithelial neoplasia [CIN I-III.]), and early cancer of the uterine cervix, with particular attention to those located in the transformation zone and endocervical canal. Since mid 1960s more and more cytology laboratories – based on pathology departments – had been set up in Hungary. In 1972, a "School of Cytotechnologists" to provide regular training for "pre-screeners" was established, and the system of "pre-screening" introduced. By the end of 1970s, the sufficient capacity to meet the demands of three-yearly cytology screening of entire eligible population was in place [5].

By the end of 1970s, more and more gynaecologists became experienced in the practice of colposcopy. In 1976, a joint deliberation by the Board of Gynaecology and Board of Oncology was issued saying that "every gynaecological examination should be performed as screening", and, "no cervical screening without cytology" [6]. C*olposcopy completed by cytology* had become the screening protocol: the smear-taking by gynaecologists as well as colposcopy had been carried out as part of a complete gynaecological examination. In fact, the gynaecologists have monopolised the cervical screening, and "*gynaecological cancer screening* instead of *"cervical screening"* had been the widely recognised practice in the country.

During the 1980s, a country-wide opportunistic "cervical screening programme" had taken place. The annual number of smears analysed exceeded one million, the clinical stage of the detected cervical cancers had favourably shifted,

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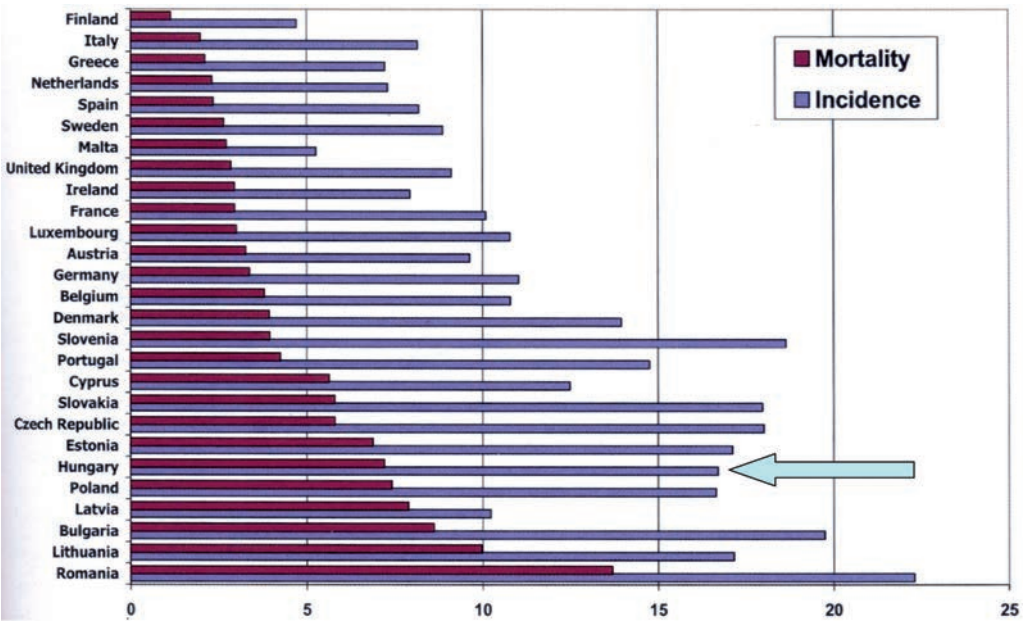

Figure 1. — Cervical cancer: incidence and mortality in the European Countries.

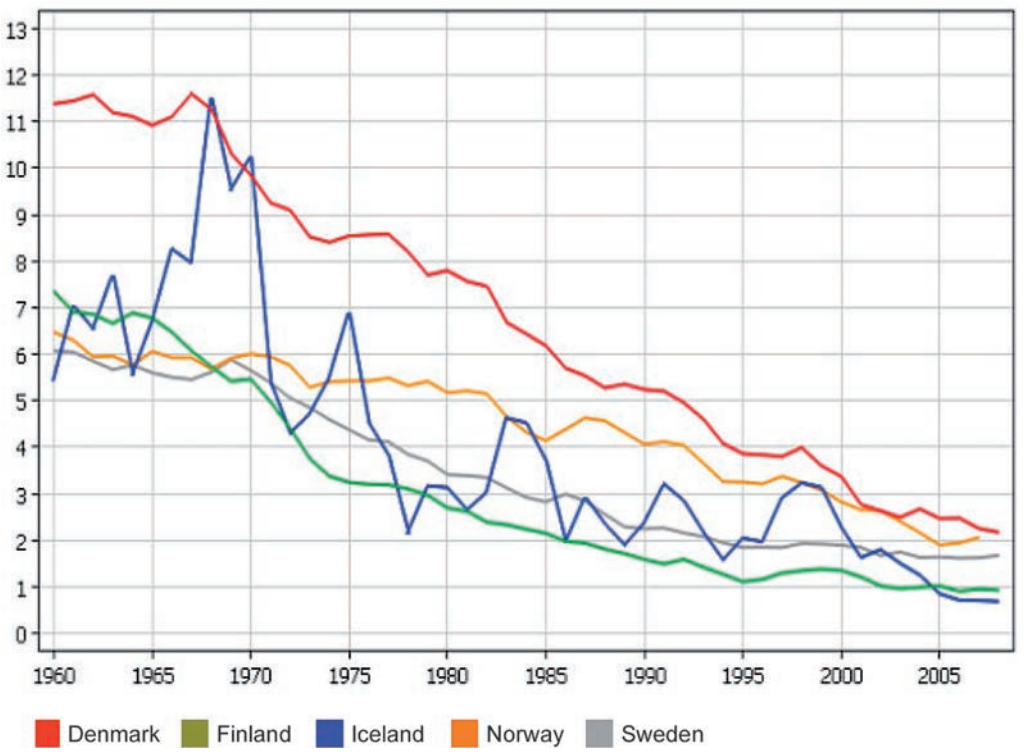

Figure 2. — Mortality from cervical cancer in the Nordic Countries, as function of intensity of screening strategy. Source: Nygård M.: "Screening for cervical cancer: when theory meets reality". *BMC Cancer*. 2011, *11*, 240.

however the mortality rates had not decreased, but did level off at a rather high level (8-10/10^5 population). It has been admitted that the "cervical screening programme" had failed [7]. The reason for failure was obviously the lack of individual identification of women to be screened: only the number of smears analysed was registered, and not the women screened. As a result, certain self-selected women (approximately 30% of the eligible ones) had been screened with unnecessary frequency, while the majority of the women had never been screened.

In the mid 1990s, as part of "secondary prevention of cancer" sub-component of the "Close the gap" programme"

co-sponsored by the World Bank, a "model" cervical screening programme was implemented on a limited scale, with the aim of introducing elements of "organized screening", as recommended by WHO/IARC and UICC [8], and to adopt the recommendation to the local needs and opportunities [9]. The pilot programme has created a favourable policy-environment for integration of organized cervical screening as a core function of the healthcare system.

In 2003, as a component of the National Public Health Programme launched by the Government, a country-wide National Cervical Screening Programme has been established, with the National Chief Medical Officer's Office in charge. All requirements of "supply side" of organised screening have been met [10]. The programme was a nation-wide, provider-initiated, invitation-based organised screening, supported by a National Screening Registry. Its objective was to three-yearly screen the asymptomatic women of 25-64 years of age by cytological analysis of cervical smears, and to refer those with abnormal (*non-negative)* cytology to gynaecological services to further diagnostic procedure including colposcopy and appropriate treatment. Notwithstanding, the gynaecological community has continued the traditional practice and they remained in the driver's seat of cervical screening. Furthermore, they insisted on a bimanual pelvic examination complemented with colposcopy, in addition to smear-taking. All the women eligible for screening received invitation letter, however, the majority of women – without waiting for invitation, or even having received it – have reported themselves for "gynaecological screening" by their "own" (private) gynaecologists, and, these screening examinations had *not* been registered by the National Screening Registry. As a result, the *coverage* of screening (i.e. proportion of women who rejected the invitation and turn up for screening outside the organised programme) has been rather high (approximately 60%), but *compliance* rates (proportion of those women who accepted the invitation, screened inside the programme, and registered by the screening registry have remained unacceptably low (8-10%), and a significant proportion of the female population has never been screened.

The analysis of the reasons for non-attendance at the offered screening showed that one of the main difficulties to overcome is the limited access to the smear-taking gynaecological service, particularly in rural areas where gynaecological services are not available locally. According to the reimbursement data provided by the National Health Insurances Fund, much more smears were taken and analysed outside as compared to inside the programme.

The question presented itself as to whether what is called "population cervical screening" was really a *health policy* and *service-based screening programme*, or a widespread clinical exercise? In the view of gynaecological community (and to certain extent in the conscience of the public at large, too) it seemed to be the latter one. There was an urgent need to reconsider and reorganise the screening practices going by the "state-of-the-art" screening strategy in Hungary, to erase the term of "gynaecological screening" form the consciousness of the women at large.

Efforts to reorganise the screening practices: the role of "health visitors"

2008 was a turning point. The Hungarian National Audit Office carried out an investigation "about the utilisation of the financial resources expended for screening programmes", and as a result they made proposals to the health government to reorganise the cervical screening programmes in line with the international recommendations, practically to apply cytology as *sole screening test,* to bring closer the provision of organised cervical screening to primary care practices; furthermore, the smears be taken by primary care personnel and non-negative cases be referred to gynaecological services for further clarification [10]. Following these recommendations, the health government (Ministry of Human Resources) decided to improve access to screening facilities by intensifying the involvement of primary care personnel, particularly the "health visitors" in the screening process.

Education and training of the health visitors

The *"health visitors"* are the public health nurses, who are ubiquitous, and qualified to provide preventive services to the female population in the country. They are professionals receiving higher education, i.e. a four-year course following the secondary high school graduation. Their activities have been organized at the primary care level, working in close cooperation with local primary care physicians. They have personal contact with virtually all invited women, and they seem suitable for taking the cervical smear, and have easy access to those who have difficulties in seeking gynaecological services [11].

Their new task as smear-takers would have remarkable implication for education and training [12]. First of all, proper communication skill is required when counselling, and providing pre-screening information, while answering questions of the women to be screened. Then, they must have some basic knowledge in epidemiology, anatomy, physiology, and pathology of cervical cancer and precursors. They have to be familiar with the theory and strategies of cervical screening, classification of smears, psychological side-effects, and ways to prevent them. They must know the referral routes to the gynaecological services. In addition, they must have skills in asking the women about their general health, provide information, and gain informed consent. Most importantly, they have to be *skilful in taking smears* from the uterine cervix and the endocervical canal, transfer of cellular material onto the glass slide, fixation and label of the sample, and its transportation to cy-

tological laboratory. Having all these in mind, a curriculum had been designed, approved, and accredited by the relevant authority. It consisted of three elements. The first phase includes a 40-hour theoretical course, presenting all the knowledge as set out above; second, a two-day session on communication with the women to be screened; and third, under supervision of a gynaecologist, a period of training in smear-taking, until they achieved the necessary skill. One is pronounced skillful enough if 30 good quality smears are obtained, as judged by a competent cytopathologist. After having completed the course, the candidates take a final examination, and receive "certificate of competency" that authorises them to carry out cervical screening in their localities according to the screening protocol. Pilot programmes of involving the "health visitors" are promising. In the near future, the knowledge and skill required by cervical screening would be included in the curriculum of graduate education and training of the would-be health visitors, and their screening activities extended nation-wide.

Discussion

By definition, *screening* aims to early detect cancer and its precursors by means of regular examination of predominantly asymptomatic individuals of appropriate age without any complains, using evidence-based screening tests. [13]. It has the potential to significantly reduce the burden of target disease in the population, and to detect malignant tumours, as well as precursor lesions *earlier* than it would be the case without screening, and to refer them for appropriate diagnostic procedure and treatment.

According to the valid "state-of-the-art recommendations", *cervical screening* means *"microscopic analysis of the sample obtained by after visualising the cervix, taken from the ectocervix, transformation zone, and endocervix*" [3]. Cervical screening reduces the incidence of cervical cancer and its precursor lesions, and mortality from cervical cancer, and improves quality of life. The degree of reduction of lesions in question seems to be proportional to the intensity of screening strategy, i.e. the age range, frequency, and interval between consecutive screening episodes (Figure 2) [1]. Cytological screening every three to five years can potentially prevent up to four out of five cases of cervical cancer, and can reduce cervical cancer incidence up to 80% at population level [14]. Such benefits can only be achieved if screening is provided in organized, population-based programmes with quality assurance at all levels [15]. Establishment of screening registries, and linkage of individual screening data with cancer registry data, taking into account appropriate data protection standards and methods, are essential tools of monitoring and evaluation.

In Hungary, gynaecologists traditionally have a key role to play in cervical screening process by taking cervical smears themselves, in addition to assessment by colposcopy and bimanual pelvic examination. In the current "gynaecological screening protocol" physical examination of breasts has been added, that is obviously not suitable for detecting "small" lumps in the breasts which is the ultimate aim of breast screening [16], however, it has been admitted by highly competent gynaecologists that the aim of a "gynaecological cancer screening" is nothing but "cervical screening" [17].

In the beginning, the screening test applied was *colposcopy alone.* Since Hinselmann first described the basic colposcopic equipment and its use establishing the foundation for the practice of colposcopy [18], this optical method was becoming widespread throughout the countries, particularly those under German influence, such as Hungary, and since the 1960s it has been widely used oversees, too. Colposcopy is a *diagnostic procedure* to examine an illuminated, magnified view of the cervix. Many premalignant and malignant lesions in this area have discernible characteristics which can be detected through the examination, allowing the colposcopist to visually distinguish normal from abnormal appearing tissue and take directed biopsies for further pathological examination [19-21]. However, when colposcopy is evaluated for primary screening, it has been accompanied by simultaneous cytology. The rationale beyond this combined testing approach is that it decreases false negative and false positive rates associated to cytology alone, and also reduces the need for call-back for repeat cytology, being used as a guide for collection of the cytology specimen [14].

The unbiased assessment of the accuracy of colposcopy requires the independent verification with a gold standard, which usually relies on histology. Without histological confirmation, colposcopically negative cases are very often considered as truly negative, and in case of endocervical location of the squamo-columnar junction, or glandular cervical lesions, colposcopy may be really false negative. The sensitivity of colposcopy directed biopsy for CIN2+ in women with satisfactory colposcopy was 57-81% [22, 23].

Constraints limiting the application of colposcopy to primary screening include its high costs relative to cytology, the availability and accessibility of trained colposcopist having long enough experience to acquire expertise in recognition of specific patterns in the epithelium of surface of cervix, and – last but not least – the lower ability to detect endocervical lesions. The expert colposcopist may be able to predict the histological diagnosis quite accurately, but in general, the coloscopic-histological correlation is only moderate [24].

Colposcopy continues to be used routinely as standard gynaecological *diagnostic* throughout Europe provided the availability of the instruments and trained colposcopists. The most common reason for referral of women for colposcopy is abnormal cervical cytology discovered as a result of cytological screening. It is important that all women with high-grade abnormalities (CINII-III) be referred im-

mediately for diagnostic colposcopy. Colposcopy is widely used as a clinical diagnostic procedure, but definitely *not recommended as a primary screening tool, particularly not as method for population-oriented mass screening.*

The other element of "gynaecological screening" is the *bimanual pelvic examination*, i.e. palpation to assess the position, size, mobility, lumpiness, tenderness of uterus, as well as the annexes. However, the palpation is obviously not sensitive enough to recognise any lesions of endometrial layer of uterine corpus and the ovaries. This procedure might be clinically justified, but does not bring closer at all to the aims of cervical screening, and does not fit in the public health agenda [25].

The current "gynaecological cancer screening" protocol prescribes a complicated multistep diagnostic procedure performed by medical specialist [16]. Conversely, a screening test as a public health procedure, by definition, should be simple, i.e. easy to perform and interpret acceptable, accurate, reliable, sensitive, and specific [13]. It follows that to link a mass screening examination as a public health measure to clinical diagnostic setting, and to a complex clinical examination, is an obvious fallacy and nonsense.

Gynaecologists have an important, nothing to replace with, role in "second step" of screening: to clarify a case with abnormal cytology, women need to be referred to a gynaecologist who can exclude or confirm the suspicion of malignancy suggested by the cytological examination, in other words, he/she establishes the diagnosis. However, "screening" which takes place opportunistically in the gynaecological practice, on the initiative of the individual woman or her doctor, by colposcopy and pelvic examination in clinical settings, should be discouraged. (Of course, this recommendation does not contradict the necessity of the periodical gynaecological check-up which can be reasonably advised to and expected from each health-conscious woman). Bimanual pelvic examination must not be undertaken as a routine part of sample-taking in asymptomatic women. Such "screening practice" results in high coverage in self-selected individuals who are screened too frequently; at the same time in a low coverage of other population groups. Its effectiveness is limited and cost-effectiveness is poor.

The task of smear-taking for cytology and referring those with abnormal cytology to gynaecological services fits very well in the traditional preventive role of "health visitors". They can personally contact each woman who has been invited by the National Screening Registry, and encourage them to accept the offered screening. They can pay particular attention to those who have never attended a screening test, and to the less educated ones of lower socioeconomic status. They can easily provide pre-screening information on both the benefits and potential harms of screening, while assisting them to gain informed decision to participate. They can arrange mutually suitable appointment. Most importantly, they can take the cervical smears for cytology, and send it to cytology laboratories. After acquiring the test results, they can reassure the woman if the test is negative, and, in case of non-negative result, they can explain what "abnormality" means, and must refer the woman to gynaecological services for further examination (including colposcopy). Last but not least, they can offer psychological support to those who receive "bad news".

Ultimately, one can conclude that for the time being, cytology has remained the standard method of population screening for cervical cancer in Hungary. However, not only the strengths but also the limitations of cytology for cervical cancer screening must be recognised.

As far as the value of conventional cytology is concerned, there is room for criticism. Cytology is a subjective test, and in programmes without quality assurance, it is impossible to achieve and maintain its proper performance. Cytology is labour intensive; a great number of trained prescreener cytotechnologists and cytopathologists are required to be employed to meet the needs of a country-wide mass screening. Despite the low cost of consumables, high-quality cytology is expensive in absolute terms and may not be the most cost-effective option for screening [26]. Furthermore, as opposed to conventional cytology, liquid-based cytology has logistical and operational advantages (interpretation at higher speed, lower rate of unsatisfactory smears, and possibility of ancillary molecular testing using remnant fluid), but is more expensive, and neither more sensitive nor more specific than conventional cytology with respect to detection of histologically confirmed high-grade CIN [27].

Finally, the needs of populations vaccinated against HPV-16/18 have to be taken in consideration. In this case, we should anticipate that the positive predictive value (PPV) of cervical screening will be reduced because there will be fewer high-grade lesions among women with cytological abnormalities. It is therefore rational to develop multiple modalities for cervical cancer prevention, including methods that achieve similar or better screening performance than cytology alone, but also meet the demands of underserved populations, such as low cost, the need for fewer visits (i.e. cytology, diagnostic colposcopy, and treatment) in each screening cycle, and fewer interventions in a lifetime due to a grater negative reassurance of a single screening intervention [28].

Conclusion

The current state of conventional, morphology-based cervical screening as a public health measure calls for a "shift of paradigm of screening" for the sake of the female population. The insistence by the gynaecological community on their "historical role" seems to be a major obstacle. The "bad habits die hard". The notion of "gynaecological cancer screening" needs to be dismissed forever (because of negative psychological connotation of mentioning "can-

cer"), and instead become "cervical screening" which should come into general use. It is hoped that higher compliance with the offered screening would be attained through the involvement of the country-wide network of health visitors into the organized screening, and the ultimate aim of cervical screening programme: the improvement of women's health in the country be achieved in Hungary.

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Retrospective analysis of the survival benefit of chemotherapy for recurrent or advanced epithelial ovarian carcinoma in patients previously treated with paclitaxel plus platinum-based chemotherapy

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Summary

Aim: The outcomes of treatment for women with recurrent or advanced epithelial ovarian carcinoma previously treated with paclitaxel plus platinum-based chemotherapy were analyzed. *Materials and Methods:* Retrospective analysis was performed in a total of 65 series of treatments provided for 35 patients with a history of paclitaxel plus platinum-based chemotherapy. The chemotherapy regimens used were classified into the following four types for analysis: conventional paclitaxel plus carboplatin therapy (TC arm), pegylated liposomal doxorubicin-containing regimens (PLD arm), CPT-11-containing regimens (CPT-11 arm), and others. Disease-control rates (DCRs) were compared and subjected to univariate analysis. Progression-free survival (PFS) was determined from the date of the first cycle of each chemotherapy with the Kaplan-Meier method, and comparisons were performed using the log-rank test. *Results:* DCR was 80%, 71%, and 26% for the TC, PLD, and CPT-11 arms, respectively. The median PFS was 286, 372, and 76 days for the TC, PLD, and CPT-11 arms, respectively. There was no discernible difference in PFS between the TC and the PLD arm. In contrast, PFS of the CPT-11 arm was significantly shorter than that of the TC and PLD arms. In addition, three of seven (42.9%) treatments in the PLD arm maintained a progression-free period for longer than one year, while only one of 25 (4%) treatments in the TC arm maintained a progression-free period for more than one year. *Conclusions:* The PFS of PLD is similar to that of TC. PLD-containing regimens might have a potential benefit with a higher PFS over one year than the TC regimen.

Key words: CPT-11; Disease control rates; Ovarian carcinoma; Pegylated liposomal doxorubicin; Progression-free survival.

Introduction

Recurrent ovarian carcinoma is generally incurable [1], and, therefore, several treatments including chemotherapies, selected surgery, and radiation are combined for each patient. However, the effects of these treatments on progression-free survival (PFS) have not been evaluated. Therefore, the outcomes of each treatment for women with recurrent or advanced epithelial ovarian carcinoma previously treated with paclitaxel plus platinum-based chemotherapy were evaluated.

Materials and Methods

All women with a histologically confirmed diagnosis of epithelial ovarian cancer who were referred to the present center from January 2007 to December 2011 were included in a database of treatment and outcome variables. The charts of patients who progressed while receiving or after completion of at least three cycles of paclitaxel plus platinum-based chemotherapy or discontinued treatment early because of toxicity were reviewed to update their follow-up. The follow-up period was ended in December 2012. All patients had measurable lesions that conformed to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, and tumor response evaluation was performed according to the RECIST v1.0 guidelines [2].

Whether and how a patient should be treated in cases of relapse were up to the investigator's discretion, and the documented therapies were independently assigned to the following groups. For platinum-resistant patients whose recurrence was documented within six months of platinum-based therapy, the regimen of chemotherapy was a non-platinum regimen as second-line treatment. The chemotherapeutic choices included pegylated liposomal doxorubicin (PLD), irinotecan (CPT-11), gemcitabine, and docetaxel. For platinum-sensitive patients whose recurrence was documented for more than six months after the completion of platinum-based therapy, platinum-based chemotherapy was used again. Among carefully selected patients, secondary surgery with complete cytoreduction (no visible residual disease) or irradiation was also performed.

The disease-control rate (DCR) was defined as either tumor response (CR/PR) or stable disease (SD). DCR was compared and subjected to univariate analysis. PFS was determined from the

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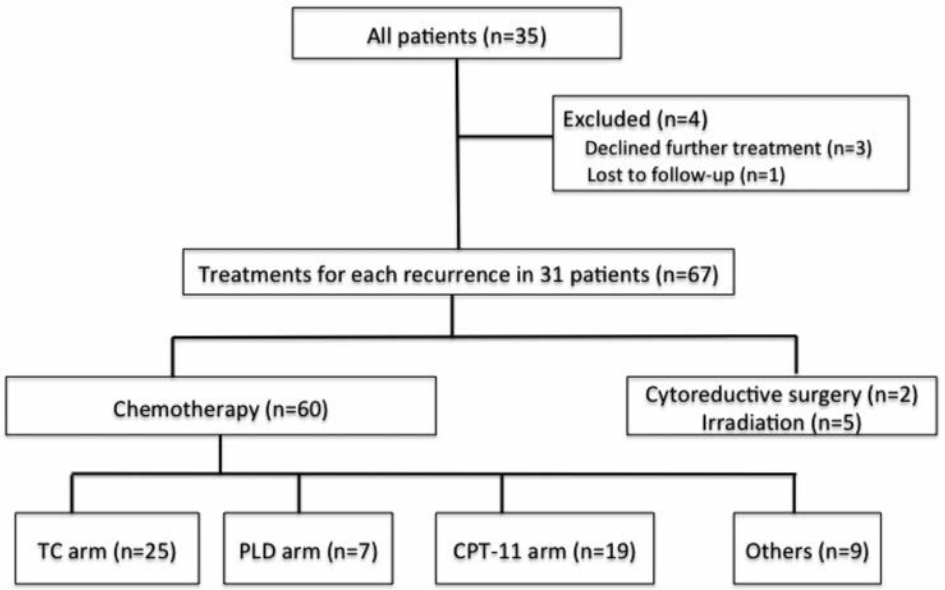

Figure 1. — Study design. TC arm, carboplatin and paclitaxel; PLD arm, pegylated liposomal doxorubicin-containing regimens; CPT-11 arm, CPT-11-containing regimens.

Table 1. — *Patients' clinical characteristics (n=35).*

Characteristic			No. (%)
Mean age	Years		60.9
	Range		(41-83)
Stage	I		5 (14)
	II		1 (3)
	III		22 (63)
	IV		7 (20)
Tumor histology/cytology		Serous	24 (69)
		Mucinous	3 (9)
		Endometrioid	0 (0)
		Clear cell	6 (17)
		Others	2 (6)

Table 2. — *Treatment response, duration of exposure, and discontinuation of treatment.*

	TC (n=25)	CPT-11 (n=19)	PLD (n=7)	Others (n=9)
DCR	80%	26%	71%	33%
Median PFS (days)	286	71	372	78
PFS > 1 year	4%*	0%	42.9%*	0%
Duration of exposure (days)	117 (3-175)	62 (5-210)	167 (42-372)	74 (19-127)
Discontinuation due to AE	2 (8%)	2 (10.5%)	0	0

TC: carboplatin and paclitaxel;
PLD: pegylated liposomal doxorubicin-containing regimens;
CPT-11: CPT-11-containing regimens;
DCR: disease-control rate; PFS: progression-free survival; AE: adverse event.
*$p < 0.05$.

date of the first cycle of each chemotherapy to first disease progression and, thereafter, from one progression to the subsequent one or the last contact date that the patient was still known to be progression-free. The Kaplan-Meier method and the log-rank test were used to analyze PFS, and a Bonferroni correction was then used for comparisons of multiple groups. Differences were considered significant if the *p*-value was < 0.05. Statistical analysis was performed using MedCalc version 12.7.5.

Results

Eighty-nine patients with histologically confirmed epithelial ovarian cancer were referred to the present center from January 2007 to December 2011. In 35 patients, at least one relapse was reported after first-line therapy (Figure 1). Three patients refused further treatment, and one patient was lost to follow-up. Information on subsequent therapies after the first recurrence was evaluable in 31 patients. The patients' characteristics are shown in Table 1. A total of 67 treatments was documented. These treatments comprised of different modalities such as chemotherapy (n=60, 89.6%), surgery (n=2, 3.0%), and radiotherapy (n=5, 7.5%). The most commonly used chemotherapeutic regimen was a combination of paclitaxel plus platinum-based chemotherapy (n=25, 41.7%; TC arm), followed by CPT-11 containing regimens (n=19, 31.7%: monotherapy with CPT-11 n=2, combination of CPT-11 and docetaxel n=16, combination of CPT-11 and platinum n=1; CPT-11 arm), and PLD-containing regimens (n=7, 11.7%: monotherapy with PLD n=5, combination of PLD and carboplatin n=2; PLD arm). Two patients with platinum-resistant recurrent ovarian cancer underwent secondary cytoreductive surgery. In one case, splenectomy was performed for isolated splenic metastasis. The patient had a 179-day remission before the next recurrence of her disease. In the other case, for a local recurrence in the right retroperitoneal space and brain metastasis in the cerebel-

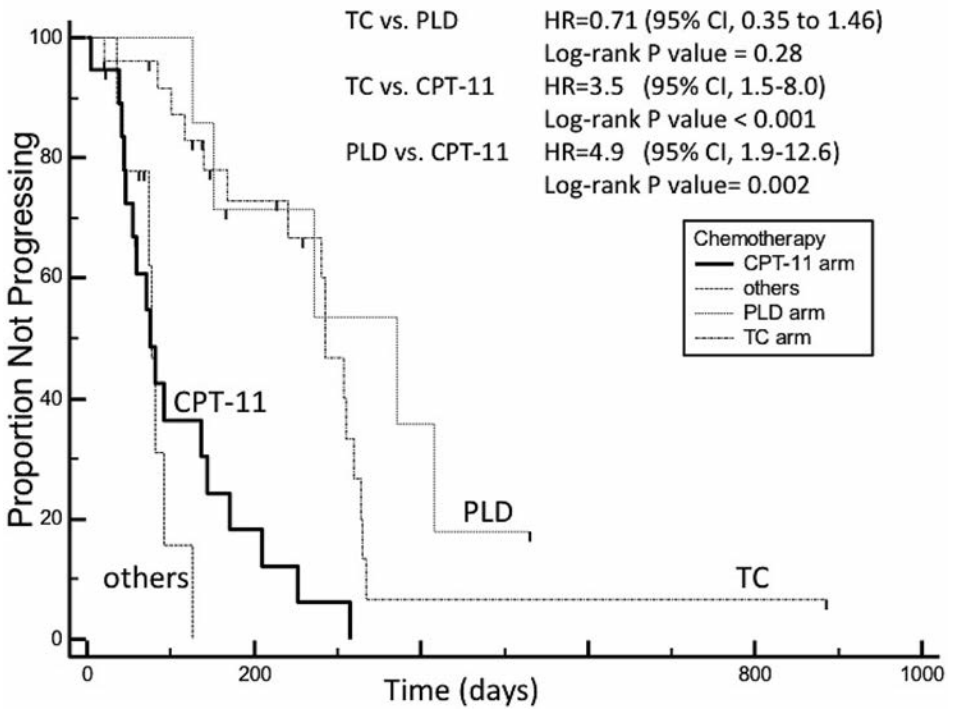

Figure 2. — Kaplan-Meier estimates of progression-free survival (PFS). HR: hazard ratio; TC arm: carboplatin and paclitaxel; PLD arm: pegylated liposomal doxorubicin-containing regimens; CPT-11 arm: CPT-11-containing regimens.

lum, excision of tumor from the pelvic peritoneum and of the brain metastasis was performed. She survived with no evidence of recurrent disease (CR) for about 32 months. These two cases of cytoreductive surgery were excluded from the analysis due to their small number. Five cases of irradiation due to palliative administration for recurrent ovarian cancer were also excluded.

DCR was 80% for the TC arm (CR n=7, PR n=9, SD n=4), 71% for the PLD arm (CR n=1, PR n=1, SD n=3), 26% for the CPT-11 arm (PR n=2, SD n=3), and 30% for others (PR n=1, SD n=2) (Table 2). The median PFS of each chemotherapeutic regimen is shown in Figure 2. The median PFS was 286 days for the TC arm, 372 days for the PLD arm, 71 days for the CPT-11 arm, and 78 days for others. A Kaplan-Meier analysis of patients in the PLD arm and the TC arm showed no significant difference in PFS ($p = 0.28$). On the other hand, PFS was longer in patients in the TC arm than in the CPT-11 arm ($p < 0.001$). PFS of patients in the PLD arm was also significantly longer than that of patients in the CPT-11 arm ($p = 0.002$). Exploratory analyses examining the impact on PFS of the number of previous lines of chemotherapy, histologic classification of tumor cells, and chemotherapeutic regimen were performed using Cox proportional hazards regression. PFS was significantly shorter in the TC arm and the PLD arm than in the CPT-11 arm in the multivariate Cox regression model (data not shown). In addition, with the limitation inherent from the small numbers, 42.9% (n=3 of 7) in the PLD arm significantly maintained a progression-free period for longer than one year, while only 4% (n=1 of 25) in the TC arm maintained a progression-free period for more than one year ($p > 0.05$) (Table 2). Treatment discontinuation because of adverse events (AEs) occurred with four treatments (28.6%), two of which occurred in the TC arm and the two of which occurred in the CPT-11 arm.

Discussion

Once diseases recur, patients with relapses six months after completion of initial platinum-based therapy are considered platinum/taxane-sensitive. Chemotherapy with platinum is a standard treatment, and patients were treated with TC or carboplatin and PLD [3-5].

Patients with no response or responses lasting less than six months are platinum/taxane-resistant and are best treated with agents that lack cross-resistance to the TC regimen. Most common chemotherapeutic choices for platinum/taxane resistance included PLD, CPT-11, and gemcitabine [6-10].

In this trial, PFS was similar between the PLD and TC arms, and PFS was significantly longer for the TC arm/PLD arm than for the CPT-11 regimen or other regimens. In the CALYPSO Trial, PFS for PLD with carboplatin (CD) was significantly superior to that of TC [6]. The PLD arm in this study consisted of five monotherapy and two CD regimens. In the present study, though most of the cases were monotherapy, the PLD arm had prolonged PFS, as did the

TC arm. Thus, on the basis of a median PFS of 372 days, PLD performed better than expected in terms of PFS.

Gorden *et al.* reported that a median PFS of PLD monotherapy in patients with epithelial ovarian carcinoma that recurred after or that did not respond to first-line platinum-based chemotherapy was 19.1 weeks, and the overall response rate for PLD was 19.7% [7]. In Japan, Katsumata *et al.* reported that the median time to progression of PLD monotherapy in Japanese patients with Müllerian carcinoma previously treated with platinum-based chemotherapy was 166 days [11].

With respect to PFS of longer than one year, PLD was superior to TC. One of the reasons was that the duration of treatment was longer in the PLD arm than in the TC arm (167 *vs.* 117 days in the median duration of exposure to chemotherapy, respectively) due to lower toxicity.

As for toxicity, in the HeCOG trial, the rate of discontinuation due to toxicity was significantly higher in the paclitaxel group (13.5% in CP *vs.* 3% in CLD, $p = 0.020$) [12]. In the CALYPSO trial, fewer patients discontinued treatment early for toxicity with PLD than with TC regimens (6% *vs*. 15%; $p < 0.001$) [6]. In the present study, PLD was used for extended periods of time with very minimal toxicities. Several RCTs failed to show an improvement of survival for patients with recurrent ovarian carcinoma who received extended therapy [13]. However, some reports suggested that continuous PLD treatment in patients who achieved a response to PLD might delay time to disease progression. Collins *et al.* reported that two patients were maintained on PLD with stable disease for 18 and 34 months, respectively [14]. These cases demonstrated that PLD can be used for extended periods of time without cardiotoxicity.

Although a small number of cases was presented, the present data support the clinical efficacy and tolerability of PLD-containing regimens for the treatment of recurrent ovarian cancer. PLD can be used for extended periods of time with very minimal toxicities. These characteristics of PLD allow extended chemotherapy and might delay time to disease progression. PLD may be a promising agent in recurrent ovarian carcinoma.

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The antibody-based CA125-targeted maintenance therapy for the epithelial ovarian cancer: a meta-analysis

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Summary

Objectives: To assess the effect and toxicity of CA125-targeted antibody used as maintenance therapy for advanced epithelial ovarian cancer (EOC). *Materials and Methods:* Two reviewers searched PubMed, Medline, Embase, VIP databases, and the references of selected articles for randomized controlled trials comparing maintenance CA125-targeted antibody treatment with placebo/observation. One -, two-, three-, and five-year overall survival (OS) and progression free survival (PFS) were collected. Incidence and severity of adverse events were extracted. Meta-analysis of combined risk ratio (RR) for OS , PFS, and toxicity were conducted. *Results:* Four trials including 1,259 women were identified. Meta-analysis showed the combined RR was 1.02 (95% CI, 0.85−1.22) for three-year OS and 0.98 (95% CI, 0.70−1.39) for the three-year PFS. This review found that abagovomab and oregovomab caused toxicity no more than placebo. *Conclusions:* CA125-targeted antibody used as maintenance therapy alone is not more effective than placebo but they were safe as maintenance therapy.

Key words: CA125-targeted antibody; Epithelial ovarian cancer; Maintenance therapy; Meta-analysis.

Introduction

Epithelial ovarian cancer (EOC) has the highest mortality rate of all gynecological cancers. There were an estimated 21,880 new cases resulting in 13,850 deaths in 2010 [1]. EOC accounts for approximately 90% of all cases of ovarian cancer and debulking surgery following six courses of platinum-based chemotherapy is the standard first-line treatment which results in complete clinical remission (CCR) in up to 75% of cases [2]. Despite high response rates, the recurrence and mortality rates are high [3, 4]. Proofs have shown that increasing cycles of chemotherapy is of little survival benefit but increases the adverse side effects [5, 6]. In 2003, Southwest Oncology Group (SWOG) reported a phase-III randomized trial of 12 versus three months of maintenance paclitaxel in patients with advanced ovarian cancer after complete response to platinum and paclitaxel-based chemotherapy [7]. Data showed the median progression-free survival (PFS) was 21 and 28 months in the three-cycle and 12-cycle paclitaxel arms, respectively. The p value was less than 0.005 in favor of the 12-cycle arm. The trial discontinued because of the protocol-specified early termination boundary of $p = 0.005$. As of the date of study closure, there was no difference in OS between the treatment arms. However, this result was encouraging to evoke a number of studies about maintenance therapy for EOC with different drugs and agents. Maintenance or consolidation chemotherapy for advanced EOC refers to the therapy given after the women have achieved CCR or pathological complete remission (PCR) following debulking surgery and induction chemotherapy[7, 8]. Currently the effectiveness of maintenance chemotherapy has been assessed but there is insufficient evidence to prove any drug is more beneficial than observation alone [9-12]. Maintenance radiotherapy may improve the five-year PFS [13, 14], however, because of the intolerable side effects, it is rarely recommended .

Immunotherapy is one of the novel therapeutic strategies for ovarian cancer. It aims to induce or enhance active immune responses directed towards the tumour and to consolidate anti-tumour effects of standard therapy, delay, and possibly prevent progression of disease. Within the last few years, different immunotherapies based on tumor-specific antibody, immunogenic peptides or vaccines have been developed [15]. CA125, also known as MUC16, is a large membrane-associated mucin protein which is over expressed in more than 80% of EOC. The soluble molecule secreted in patient blood is used as a marker for tumor identification and progression. Due to its poor immunogenicity, the host organism is not able to mount an adequate immune response against it alone. When the targeted antibody binds to the CA125, the complex can bind to the antigen-processing cells more readily than CA125 alone. During the past 20 years, many phase I/II clinical trials had studied the CA125-targeted antibodies like oregovomab,

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ACA125, and abagovomab in newly diagnosed or recurrent advanced ovarian cancer, which showed that they could cause specific immune response resulted in longer survival [16-21]. These trials evoked to imagine how they would act when used as the maintenance therapy. Further more, it is important for the gynecologists and women with EOC to assess the potential benefits and adverse effect of CA125-targeted antibodies, however, currently there have not been any systematic reviews published on this topic.

This systematic review aimed to evaluate the effectiveness, toxicity, and impact on the quality of life (QoL) of antibody-based CA125-targeted immunotherapy as maintenance therapy for EOC.

Materials and Methods

Eligibility criteria

Women with EOC, fallopian tube cancer (FTC) or primary peritoneal cancer (PPC) who have achieved CCR after debulking surgery and first-line chemotherapy. The patients were ≥ 18 years and have no other concurrent malignancies. The study design was a randomized controlled trial comparing maintenance CA125-targeted antibody treatment with observation, placebo or other treatment. Maintenance CA125-targeted antibody combined with the other treatment versus the other treatment alone is also included. The PFS rate, OS rate, incidence and severity of adverse events or QoL score were the primary or secondary outcomes of the original studies.

Searches

Two reviewers searched MEDLINE (from 1948 to 2014), EMBASE (from 1980 to 2014) , PubMed (up to October 2014), and VIP (1989 to October 2014) independently. It was designed to identify all published trials in English or Chinese.The aforementioned databases were searched using the keywords: immunotherapy, bioimmunotherapy, CA125, MUC16, oregovomab, abagovomab, ACA125, ovarian cancer, and maintenance/consolidation therapy . VIP was searched using the same keywords in Chinese. In addition, the authors reviewed the references of selected articles to identify studies missed through our databases searching. They also searched the relative websites for ongoing trials.

Two reviewers scanned the titles and abstracts from the initial search to exclude those articles which did not meet the eligibility criteria. Then the full text of potentially relevant studies were obtained and assessed by both review authors independently. Any disagreements were resolved through discussion with a third review author.

Data collection and analysis

The authors extracted OS and PFS after one, two, three, and five years from the included studies. The incidence and severity of adverse events such as nausea-vomiting, diarrhea, rash, back pain, myalgia, arthralgia, and flu-like syndrome were also abstracted. The published QoL scores were collected. The authors pooled the results of similar trials into a meta-analysis. Revman 5.2 was used to conduct the meta-analysis and calculate the combined RR and its 95% confidence interval (CI) for OS, PFS, and adverse events.

The authors also assessed the risk of bias of each trial in terms of randomisation process, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other possible sources of bias and classified them as low , high or unclear risk according to the guidelines of Cochrane Handbook for Systematic Reviews of Interventions [22].

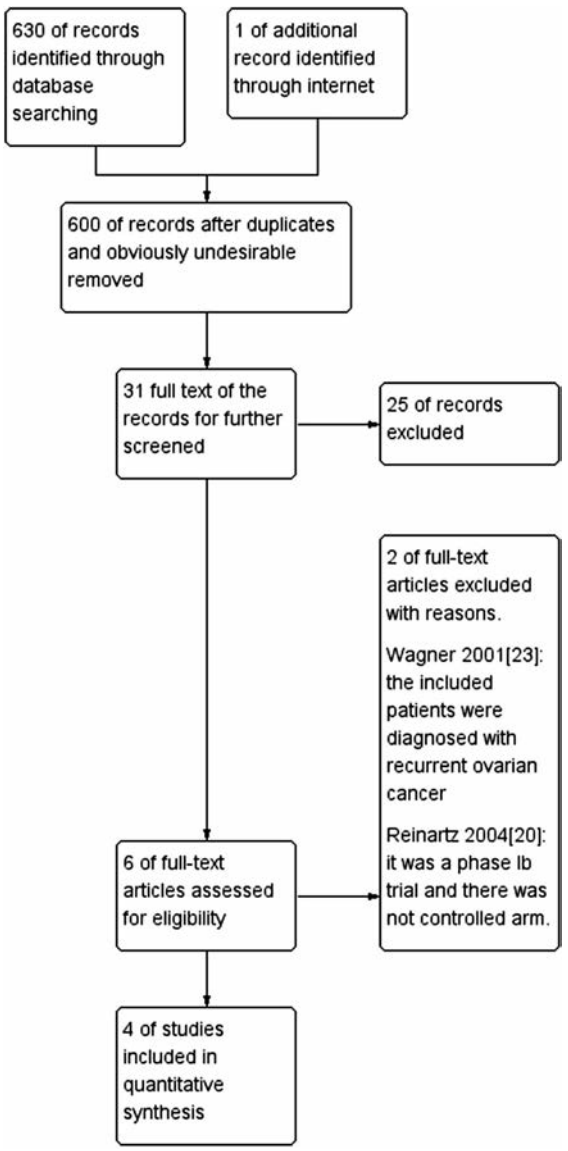

Figure 1. — Flowchart of studies screening.

Results

Data search and quality of the included studies

The search identified 631 trials and initially 600 were excluded because of duplication and obvious ineligibility after reading the titles and abstracts. Full text were obtained for the remaining 31 trials for further scrutiny and 25 ineligible trials were excluded. Two trials [20, 23] were initially identified as potentially eligible for inclusion but were subsequently found to be ineligible and therefore excluded (Figure 1). In the end, four trials (1,259 patients) were included in this review. Three trials compared oregovomab [3, 24, 25] with placebo and the other one compared abagovomab [26] with placebo. Berek *et al.* 2008 [24] is a five-year follow-up survey of Berek *et al.* 2004 [3]. Berek *et al.* 2009 [25] re-

Table 1. — *The included studies of CA125-targeted antobody as maintenance therapy for EOC.*

Study	Study Design	Patients	Outcome	Duration of follow-up	Comment
Berek *et al.*, 2004 [3]	RCT Oregovomab *vs.* placebo (1:1)	n=145 EOC of Stage III/IV achieved CCR Median age: 60 in treatment 62 in placebo group	Median TTR was 13.3 months for oregovomab and 10.3 months for placebo (p = 0.71).	Insufficient information	
Berek *et al.*, 2008 [24]	RCT Oregovomab *vs.* placebo (1:1)	n=145 EOC of Stage III/IV achieved CCR Median age: 60 in treatment 62 in placebo group	Median survival was 57.5 months for oregovomab and 48.6 months for placebo (p = 0.28)	5 years	A 5-year follow-up survey of Berek 2004
Berek *et al.*, 2009 [25]	RCT Oregovomab *vs.* placebo (2:1)	n=371 EOC of Stage III/IV achieved CCR Median age: 58.8 in treatment 59.6 in placebo group	Median TTR was 10.3 months for oregovomab and 12.9 months for placebo (p = 0.29).	5 years	The same trial of Berek 2004
Sabbatini 2013 [26]	RCT Abagovomab *vs.* placebo (2:1)	n=888 EOC/FTC/PPC of Stage III/IV achieved CCR Median age: 56.3 in treatment 56 in placebo group	HR of RFS was 1.099 (95%CI, 0.919 to 1.315) HR of OS was 1.150 (95%CI, 0.872 to 1.518).	24 months after random assignment of the last patient.	

Abbreviations: RCT: randomized controlled trial; EOC: epithelial ovarian cancer; FTC: fallopian tube cancer; PPC: primary peritoneal cancer; TTR: time to relapse; RFS: relapse free survival HR: hazard ratio; OS: overall survival.

ported the same trial but the included subjects were 226 more than Berek *et al.* 2004 and the primary end point was time to relapse. The baseline of the four studies was balanced and there was no significant heterogeneity between them (Table 1). All the four studies were randomly allocated and used blinding method. None of the trials have any attrition bias or reporting bias so the included studies had low risk of bias.

Effect of CA125-targeted antibody

Sabbatini *et al.* [26] reported the median estimated time to recurrence was 403 days in abagovomab group and 402 days in placebo group. At the end of the double-blind observation period, the hazard ratio (HR) of OS for the treatment group was 1.150 (95% CI, 0.872−1.518; p = 0.322). Berek *et al.* [3, 24, 25] reported the median survival was 57.5 months for oregovomab and 48.6 for placebo but the p-value was 0.28. The median time to relapse was 10.3 months (95% CI, 9.7− 13.0 months) for oregovomab and 12.9 months (95% CI, 10.1−17.4 months) for placebo (p = 0.29). Data from 1,033 patients was combined and the RR for three-year OS was 1.02 (95% CI, 0.85−1.22), while the combined RR for three-year PFS was 0.98 (95% CI, 0.70−1.39) (Figure 2). Therefore, there was no significant benefit of using CA125-targeted antibody alone as maintenance therapy.

Toxicity of antibody against CA125

For CA125-targeted antibodies, diarrhea was the only side effect which was recorded more in the abagovomab group (p = 0.031)[26], but the meta-analysis found that the combined RR for diarrhea was 1.36 (95% CI, 0.94−1.96) so there was no significant difference between two arms regarding diarrhea, which was the same with back pain, fatigue, and arthralgia, *et al.* (Figure 3). According to Berek *et al.*'s study [25], 13.7% of oregovomab group and 18.6% of placebo group had a serious adverse event but there was not significant difference between them (p = 0.218). Berek *et al.* [3] used European Organization for Research and Treatment of Cancer Quality of Life Questionaire C30 (EORTC QLQ-C30) to assess the overall health and overall QoL and found the QoL was similar in the oregovomab group and the control group. These results indicated that the abagovomab and oregovomab were safe and had little impact on the QoL as maintenance therapy for ovarian cancer.

Discussion

This meta-analysis is based on 1,259 women from four RCTs that used different antibodies against CA125 as maintenance therapy for advanced EOC. A number of methods were employed to identify all trials to minimize

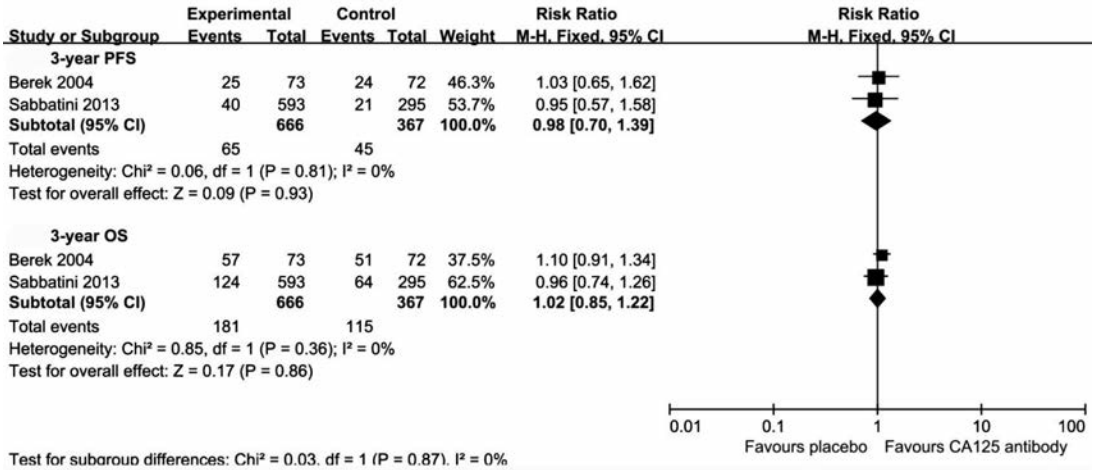

Figure 2. — Forest plot of survival analysis of CA125-targeted antibody.

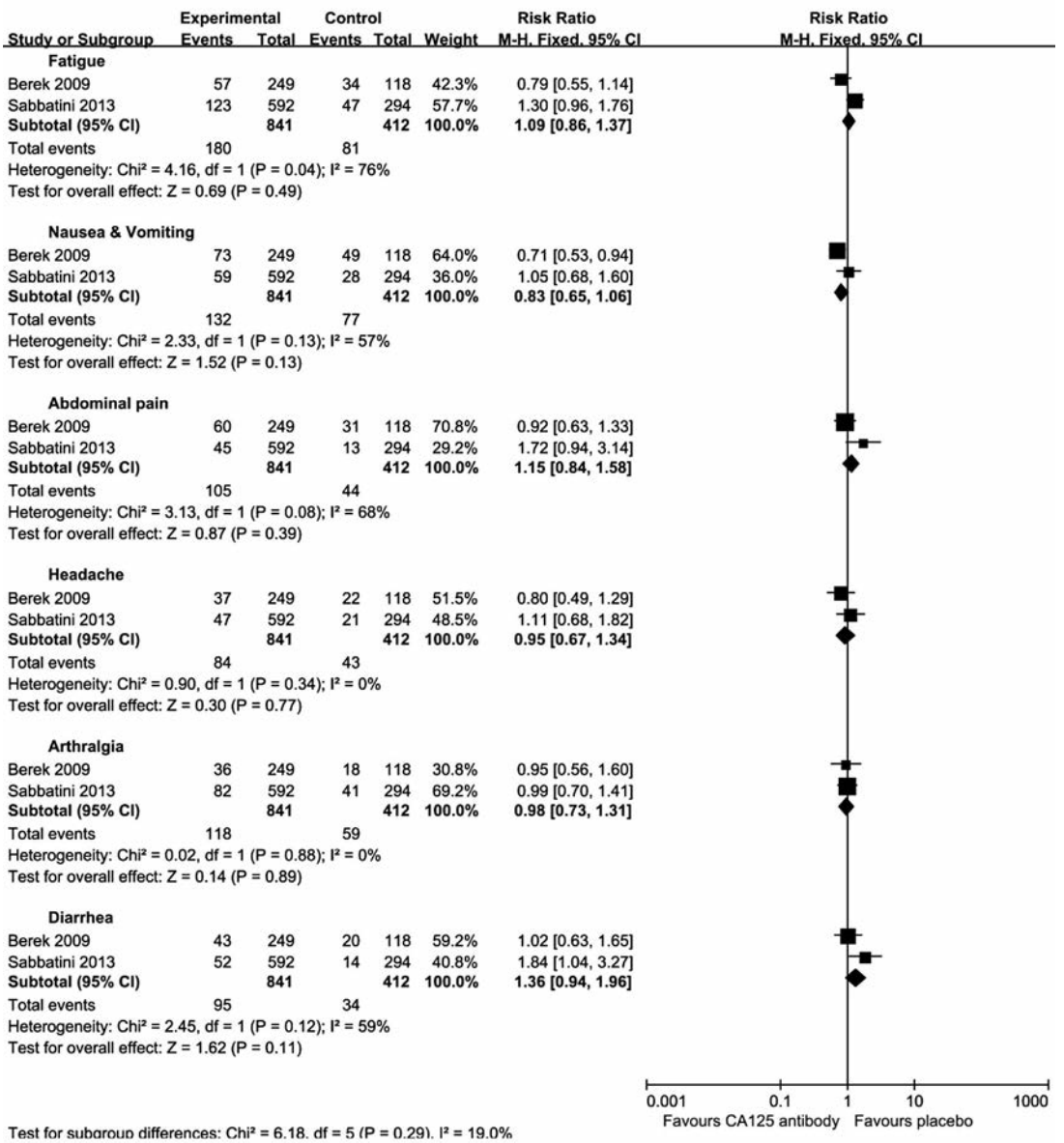

Figure 3. — Forest plot of adverse side effect of CA25-targeted antobody.

the influence of publication bias. There were no serious inconsistency, indirectness, imprecision or publication bias; this meta-analysis currently provides a reliable assessment of the average effect of CA125-targeted antibody maintenance therapy among women with advanced EOC. Overall, the quality of the evidence was moderate due to low number of studies and further researches may change the estimate.

There was no benefit of maintenance CA125-targeted monoimmunotherapy settings, the drugs, however, were well-tolerated. The adverse events were similar between treatment groups and were not life-threatening. Does it really mean maintenance CA125-targeted immunotherapy of advanced EOC is of little survival benefit? Perhaps it is too arbitrary to draw the conclusion. First of all, the included four studies had different interval between the last cycle of first-line chemotherapy and the beginning of maintenance therapy. Berek *et al.* [3, 24, 25] started oregovomab therapy within ten weeks after the last cycle of chemotherapy while Sabbatini *et al.* [26] began abagovomab therapy within 12 weeks, which was less than six months. As a consensus, the phase of CCR or PCR of platinum-sensitive cases is usually longer than six months and the prognosis and biological characteristics are totally different between platinum-sensitive and platinum-resistant cases. If maintenance therapy is initiated earlier than six months, the platinum-resistant cases would be included, which may impair the effect of the treatment.

Secondly, different studies used different criteria for judging disease relapse. Berek *et al.* [3, 24, 25] defined recurrence as identification of new intraperitoneal lesion not previously seen or a retroperitoneal lesion on CT scan greater than 2 × 2 cm. Sabbatini [26] assessed disease progression as a 20% increase in sum of longest diameters compared to baseline or appearance of any new lesions (RECIST version 1.0). Although randomization and blinding may balance the bias between the treatment and controlled arms, however the heterogeneity caused by different criteria would bring error into the meta-analysis results, especially when the weight of each study is different. As we know, second-line treatment starts usually based on an increase in serum CA125 level only, without imaging evidence for ovarian cancer because the increase of serum CA125 level occurs usually much earlier than appearance of objective disease progression. A working group of the Gynecologic Cancer Intergroup has developed definitions of CA125 progression to complement the definitions of objective disease progression in ovarian cancer [27]. It is supported that doubling in CA125 from the upper limit of normal reliably predicts objective progression. For those patients whose CA125 never fell to the normal range, a doubling from the nadir has been shown to predict progression. In an effort to address this issue in a consistent manner, the present authors suggest that the date of progression should be the date of the earlier of the two events when both the RECIT and Intergroup criteria were documented.

Braly *et al.* [17] reported a phase-II trial in which 40 patients with Stage III/IV EOC were randomized to receive a two-mg oregovomab infusion either the same day (simultaneous infusion arm) or one week after standard carboplatin-paclitaxel chemotherapy at cycles 1, 3, and 5, then quarterly for up to 11 antibody doses. Humoral immunity occurred more rapidly ($p = 0.0033$) and with greater magnitude in the simultaneous infusion arm. They came to the conclusion that the front-line chemotherapy has immune adjuvant properties when combined with oregovomab immunotherapy. Therefore combined strategies of chemotherapy with oregovomab or other CA125-targeted agents should be further studied as maintenance therapy.

Conclusions

In summary, there is insufficient evidence which adequately supports the use of antibody against CA125 as maintenance therapy alone to improve the OS or PFS for advanced EOC, however, they are safe and tolerable. The sufficient interval of CCR (> six months) before maintenance therapy and more precise unified criteria of assessing for progression may diminish the bias which would impair the results. For further study, combining the immunotherapy with the traditional chemotherapy may be a new topic.

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The outcomes of radiotherapy in patients with ovarian carcinoma

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Summary

Relapses of ovarian cancer have poor prognosis, overall survival (OS) after recurrence depends on patient's performance status, histological cell type, size and number of the relapse, and duration of the platinum-free interval. Pelvis, peritoneum, pleural effusion, liver, lung, lymph nodes, and central nervous system are the most frequent sites of relapse. The standard treatment for ovarian cancer is a combination of surgery and chemotherapy. This retrospective study aimed to describe incidence, characteristics, outcomes and prognostic factors of patients with ovarian cancer underwent radiotherapy. *Results:* In 47 with ovarian cancer underwent radiotherapy. Treatment modalities were radiotherapy 8- 56 Gy. After optimal treatment the authors observed complete remission in seven patients, and progression and/or metastases in 40 patients. The present study confirmed that patients with low advancement stage had better prognoses than patients with advanced disease, as confirmed by OS rates in groups T1 *vs.* T3 ($p = 0.066$) and T3 *vs.* T4 ($p = 0.066$). What was interesting was that the disease-free survival (DFS) in the group of patients with T3 cancer was longer than in the group of patients with T1 cancer. Time to marker progression (Ca 125) was longer in groups with FIGO Stage I *vs.* II and I *vs.* III ($p = 0.016$, $p = 0.044$), while the time to progression in FIGO Stage II cancer patients was shorter than in FIGO Stage III cancer patients. An interesting result was also obtained in the analysis of 36-month survival where a larger number of patients without the disease symptoms had T3 Stage cancer. New prospective studies, designed to include the aspects of target volumes, total doses and fraction doses, together with the use of state of the art planning techniques, and therapeutic instrumentation are required.

Key words: Cancer; Ovary; Radiotherapy; Brachytherapy.

Introduction

Ovarian carcinoma is currently the second most common cause of deaths for genital cancer in the Western countries. Standard management of ovarian carcinoma consists in combination of surgery and chemotherapy [1, 2]. Recurring ovarian carcinoma is associated with poor prognosis, with overall survival (OS) following a relapse being dependent on the overall condition of patients, histopathological type of tumor, size and number of relapse lesions, as well as time from last platinum-based chemotherapy [3].

Most common recurrence locations include pelvis, peritoneum, pleura, liver, lungs, lymph nodes, and central nervous system [3, 4]. About 70% patients with advanced-stage ovarian carcinoma respond to the first chemotherapy regimen; unfortunately, most of these patients experience disease recurrence, with median time to progression of about 18 months and median OS of about 24 months. The efficacy of chemotherapy delivered to these patients is lower than that of the first-line treatment; this means that only a small percentage of patients actually benefits from subsequent chemotherapy [5]. Currently, radiation therapy plays a less significant role, being used mainly as a next-line or palliative treatment.

The aim of this retrospective study was to assess the results of radiotherapy in ovarian carcinoma patients.

Materials and Methods

The study was conducted in ovarian carcinoma patients undergoing radiation therapy in the Department of Teleradiotherapy of the Oncology Center in Bydgoszcz between January 2009 and December 2013. The analysis covered patients with all stages of ovarian carcinoma, i.e. FIGO and TNM Stages I-IV (Table 1). The patients age ranged from 35 to 67 years. The most common cancer type as determined in histopathological assessment was adenocarcinoma and its histological variants. Lymph node metastases were confirmed in 22 patients while N0 Stage was identified in 18 patients. Tumor differentiation Grades G2 and G3 were determined in 12 and 18 patients, respectively (Table 2). Eleven patients underwent radiotherapy twice; three patients were irradiated three times while one patient underwent radiation treatment as many as five times. Radiotherapy was delivered to the pelvis in 25 patients, para-aortic lymph nodes in 12 patients, local lesions in 11 patients, and metastases in 13 patients; patients had a history of multiple surgical and/or systemic treatments.

Patients were treated in line with the accepted management standard; in most cases, this included combination treatment. Surgical treatment was undertaken in all 47 patients; of these, three

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Table 1. — *Patient's TNM and FIGO Stage.*

TNM Stage	Number of patients	FIGO Stage	Number of patients
T1	10 (21.2%)	I	7 (14.89%)
T2	13 (27.65%)	II	11 (23.40%)
T3	16 (34.04%)	III	23 (48.93%)
T4	2 (4.25%)	IV	4 (8.51%)
Tx	6 (12.7%)	X	2 (4.25%)

Table 2. — *Characteristics of patients with ovarian carcinoma.*

	Number of patients
Lymph node status	
Without lymph node involvement - N0	18 (38.29%)
With lymph node metasases - N1	22 (46.80%)
Unknown status - Nx	7 (14.89%)
Histopathological assesment	
Folliculoma	2 (4.25%)
Granulosa cell tumor	1 (2.12%)
Epitheliolid carcinoma	1 (2.12%)
Adenocarcinoma	12 (25.53%)
Adenocarcinoma endometroides	3 (6.38%)
Adenocarcinoma papillare serosum	3 (6.38%)
Adenocarcinoma serosum	1 (2.12%)
Adenocarcinoma clarocellulare	4 (8.51%)
Cystoadenocarcinoma	12 (12.53%)
Infitrartio carcinoma	3 (6.38%)
Carcinoma papillare	2 (4.25%)
Carcinoma serosum	1 (2.12%)
Carcinoma mucinosum	1 (2.12%)
Tumor differentation Grade	
Grade 2	12 (25.53%)
Grade 3	18 (38.29%)
Unknown status Gx	17 (36.17%)

patients required no adjuvant systemic treatment. The remaining patients received chemotherapy, most commonly platinum-based regimens in combination with taxoids.

Disease recurrence was experienced by 44 patients, all of whom required combination treatment including next-line chemotherapy and/or radiotherapy and/or surgery. External beam irradiation was delivered at doses of 8-56 Gy in 47 patients, including 13 patients who received palliative radiation therapy for metastases. Radiotherapy consisted of irradiation of the tumor with appropriate margin at doses of 8-20 Gy in one to five fractions as part of palliative therapy.

In patients with confirmed metastases into the lymph nodes or pelvis or patients with local recurrence, target volume most commonly included the area from the L1 segment to the obturator foramina, laterally two cm from the pelvic walls on both sides, from 1/3 of pubic symphysis to the S2/S3 intervertebral joint in anteroposterior projection so as to include para-aortic, common iliac, external iliac, internal iliac presacral, and obturator lymph nodes.

In patients with confirmed metastases into para-aortic lymph nodes, target volumes most commonly spanned from the upper edge of the 12th thoracic vertebra down to the L5/S1 intervertebral joint, including a healthy tissue margin. The treatment was delivered at total doses of 30-56 Gy, with daily fraction dose of two to three Gy using six and 15 MeV energy sources. High-dose rate (HDR) brachytherapy was performed in seven patients at doses of 10-30 Gy divided into one to four fractions.

Repeated surgery was required by 32 patients, including six patients who were subjected to at least three surgical procedures. After completion of the treatment, response was observed in seven patients, with 40 patients experiencing progression and/or distant metastases. M1 stage was developed in 31 patients; 22 died of the disease while the fates of another three patients are unknown. Follow-up visits were held at four weeks after completion of radiotherapy and then at three-month intervals. Patient follow-up lasted at least 12 months. Three patients did not report to follow-up visits; further course of their disease remains unknown.

Statistical analysis

Statistical analysis of the was performed using Statistica, version 10.0. The association between OS rate, DFS rate, progression free survival rate, and prognostic factors was estimated using Kaplan-Mayer model. Differences between categorized groups were assessed with using the log-rank and Cox-Mantel tests. Proportions of survivors at 24 and 36 months were assessed using Chi-square test. Statistical significance was considered at $p < 0.05$.

Results

OS

Parameters affecting the likelihood of survival were analyzed in the entire group, including: T-staging, FIGO classification, tumor differentiation grade G, lymph node status, treatment history including surgery, first, second, third, next-line chemotherapy, induction chemotherapy, pelvic radiotherapy, local radiotherapy, para-aortic lymph node radiotherapy, radiotherapy of metastases, and brachytherapy. Log-rank test demonstrated borderline statistical significance for longer OS in patients with tumor advancement stage T1 as compared to T3 ($p = 0.066$) and higher OS rates in patients with T3 cancer as compared to patients with T4 cancer ($p = 0.064$) (Figure 1). A trend towards higher survival rates was also observed in patients with differentiation grade G2 as compared to patients with differentiation grade G3 ($p = 0.071$). No evidence was provided for N1 Stage (presence of lymph node metastases) having a negative impact on the survival as compared to N0; likewise, no effect of distant metastases was observed.

Log-rank and Cox-Mantel tests revealed no significant effects of the second-, third- and next-line chemotherapy on OS. The next stage of statistical analysis included the assessment of the effect of radiation therapy on ovarian carcinoma patients. No effect on OS was observed for pelvic radiotherapy, para-aortic lymph node radiation therapy (Figure 2), and brachytherapy ($p > 0.05$).

A trend towards shorter OS times was observed in patient undergoing local radiotherapy as compared to patients not subjected to such treatment ($p = 0.079$), particularly with respect to long-term survival (Figure 3).

In the entire study group, statistical significance (log-rank test) was determined for the effect of metastases radiotherapy on OS; patients undergoing such treatment were characterized by longer OS times than patients not subjected to the metastases-targeted radiotherapy ($p = 0.18$) (Figure 4).

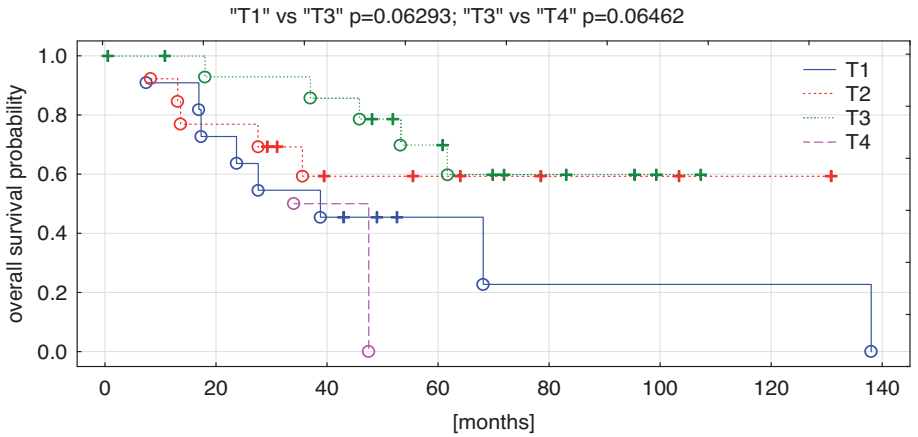

Figure 1. — Overall survival in patients with ovarian cancer in TNM stage.

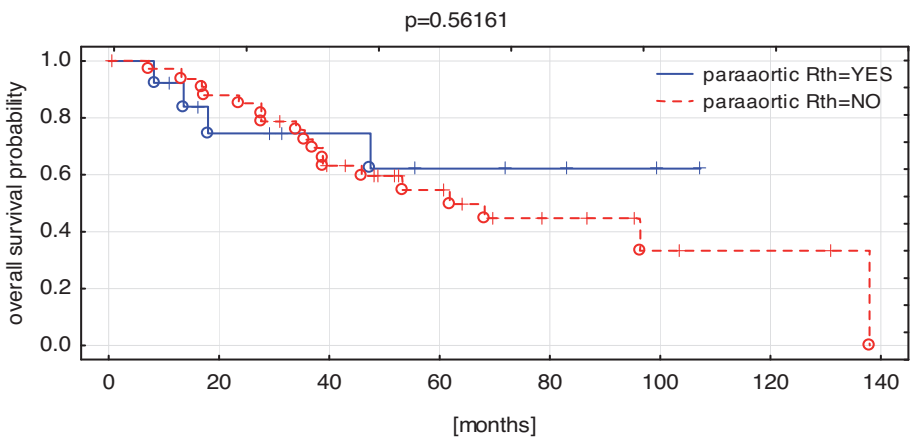

Figure 2. — Overall survival in patients with ovarian cancer with para-aortic lymph node radiotherapy *vs*. without radiotherapy.

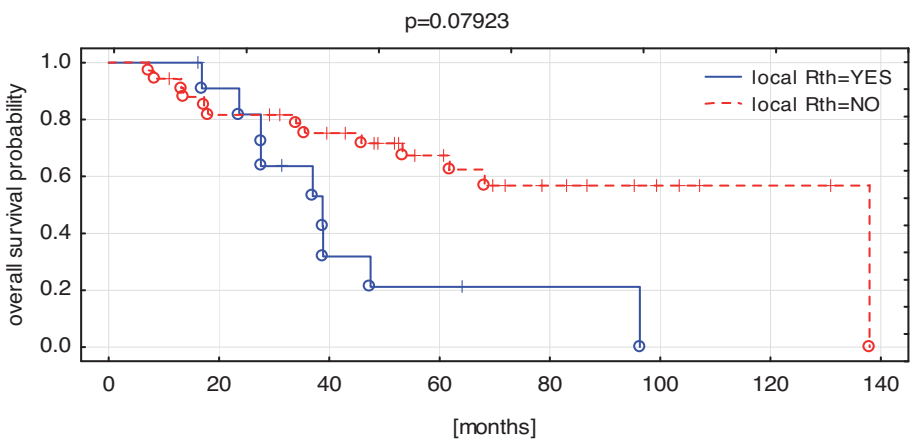

Figure 3. — Overall survival in patients with ovarian cancer with local radiotherapy *vs*. without radiotherapy.

The number of surgical procedures also affected OS at borderline statistical significance (p = 0.06, lower 95% CI limit -1.29235; upper 95% CI limit 0.04119).

DFS

The analysis of DFS time included the assessment of various parameters using the log-rank test to demonstrate that DFS was significantly longer in patients with tumor stage T3 as compared to patients with tumor stage T1 (p=0.03, lower 95% CI limit -2.62712, upper 95% CI limit 0.104210). Correlation of DFS with lymph node involvement proved to be very interesting. Statistically longer DFS times were observed in patients with confirmed lymph node metastases as compared to patients with clinical stage N0 (p = 0.046). Statistical evaluation was also performed for tumor differentiation grade confirming that

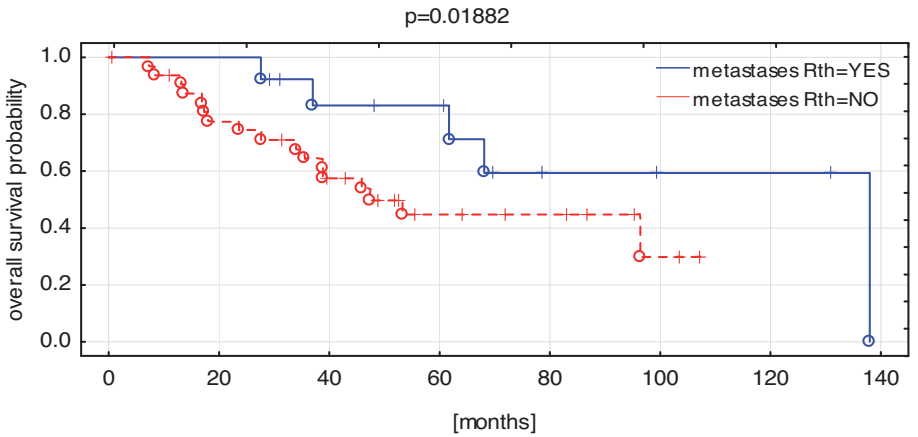

Figure 4. — Overall survival in patients with ovarian cancer with metastases radiotherapy *vs*. without radiotherapy.

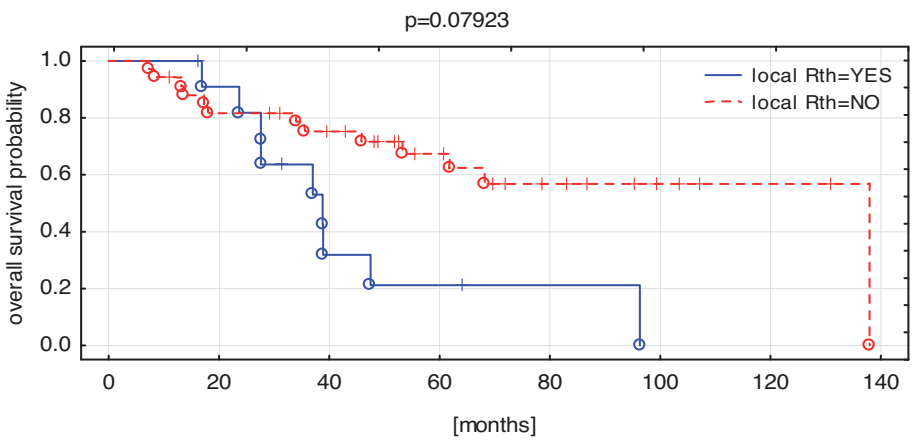

Figure 5. — Disease-free survival in patients with ovarian cancer with metastases radiotherapy *vs*. without radiotherapy.

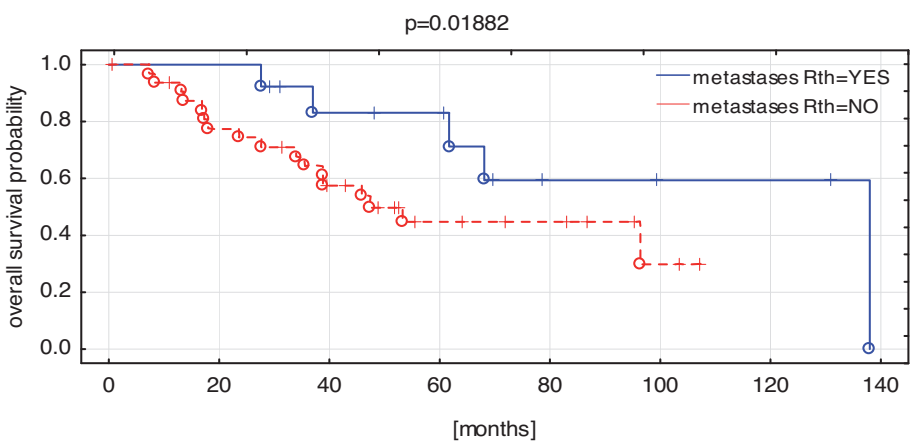

Figure 6. — Disease-free survival in patients with ovarian cancer with brachytherapy *vs*. without brachytherapy.

the DFS was longer in patients with tumor grade G2 as compared to patients with tumor grade G3 ($p = 0.27$).

With the Cox-Mantel test, a significant effect on DFS was demonstrated for first-line chemotherapy *vs*. no systemic treatment ($p = 0.007$). Patients treated with induction chemotherapy were characterized by shorter DFS as compared to patients not subjected to this treatment ($p = 0.002$).

The study did not demonstrate any effect of the second-, third-, and next-line chemotherapy on longer DFS; similarly, no effects were observed for pelvic radiation therapy, para-aortic node radiation therapy or local radiotherapy. A trend towards shorter DFS times was observed in the group of patients undergoing palliative radiation therapy for distant metastases as compared to patients not subjected to radiation therapy ($p = 0.08$) (Figure 5).

The next stage of statistical analysis included the assessment of the effect of brachytherapy on ovarian carcinoma patients. No effect on the DFS was observed ($p > 0.05$) However, curves in the Kaplan-Meier graph reveal a significant effect on local tumor control, particularly in the initial months, although no statistical significance has been reached (Figure 6). DFS time was longer in the group with minimum one surgical procedure as compared to no surgical procedures, with the difference at the border of statistical significance ($p = 0.055$).

Time until Ca125 marker progression

The next stage of the study included the analysis of time until Ca125 marker progression. The analysis revealed longer times until Ca125 marker progression in patients with FIGO stage I cancer as compared to patients with FIGO stage II cancer (p=0.016) as well as in patients with FIGO stage I cancer as compared to patients with FIGO stage III cancer (p=0.044). What is interesting, as shown in the graph, patients with FIGO stage II cancer had shorter times to progression than patients with FIGO stage III cancer, although the differences were not statistically significant).

The next step of the statistical analysis made use of the log-rank and Cox-Mantel tests to determine the effects of pelvic, para-aortic nodes radiotherapy, local radiation therapy, brachytherapy and metastases-targeted radiotherapy in ovarian carcinoma patients. Shorter times to marker progression were observed in patients receiving palliative radiotherapy for distant metastases as compared to patients who were not subjected (did not require) such treatment; the result was at the border of statistical significance (p=0.060).

None of the other parameters subject to the analysis, such as first-, second,- or next-line chemotherapy had any significant effect on the time to Ca125 marker progression.

24- and 36-month survival

Spearman's analysis was employed to analyze OS and DFS 24 and 36 months after treatment. The study showed that in the group of patients who survived 36 months without any symptoms of the disease, Stage T3 was more common than Stage T1 or T2 ($p = 0.03$). The study showed that the group of patients who survived 24 and 36 months without disease recurrence included more patients with baseline confirmation of node metastases as compared to patients with N0 Stage ($p = 0.25$ and $p = 0.25$, respectively). Further analysis revealed that in the group of patients who survived 36 months without any symptoms of the disease, Stage G2 was more common than Stage G3, and the result was at the border of statistical significance ($p = 0.062$). A borderline significance trend was observed towards a higher percentage of patients who survived 36 months without any symptoms of the disease and who had not been subjected to metastases-targeting radiation therapy (p=0.073). Statistical analyses of OS of 24 months after the baseline treatment revealed that a higher number of patients survived in the group not subjected to local radiation therapy as compared to patients who underwent local radiotherapy (p=0.01). A similar situation was observed in relation to surgical treatment: a higher percentage of patients survived 24 and 36 months after the baseline treatment if they had undergone surgery, as compared to patients not subjected to surgery ($p = 0.01$ and $p = 0.036$, respectively).

Discussion

Recent years have witnessed a return to radiotherapy as adjuvant treatment of ovarian carcinoma. That was due to the dynamic advances in the techniques involving the planning and delivery of radiation beam treatments. The goal of radiotherapy is to treat the largest possible volume within the abdominal cavity with possibly lowest risk of early or delayed radiation-induced reactions and good protection of critical organs.

Treatment of ovarian carcinoma poses a significant challenge to radiotherapist as no standards are available regarding irradiation, including total dose, fraction doses or target volume outlines. This is particularly important as the number of reports on the subject is limited and dates back to 1980s and 1990s or is based on small patient groups and/or short follow-up periods [6-12].

The present study confirmed that patients with low advancement stage had better prognoses than patients with advanced disease as confirmed by OS rates in groups T1 vs T3 ($p = 0.066$) and T3 *vs.* T4 ($p = 0.066$). What is interesting, the DFS in the group of patients with T3 cancer was longer than in the group of patients with T1 cancer. Time to marker progression (Ca 125) was longer in groups with FIGO Stage I *vs.* II and I *vs.* III ($p = 0.016$, $p = 0.044$), while the time to progression in FIGO Stage II cancer patients was shorter than in FIGO Stage III cancer patients. An interesting result was also obtained in the analysis of 36-month survival where a larger number of patients without the disease symptoms had T3 Stage cancer.

The obtained results regarding cancer staging could have been affected by the number of patients in the study group; however, in the present Author's opinion, the difference in management protocols for patients with low FIGO and TNM stage cancers and advanced cancer patients might be of importance here. In addition, ovarian carcinoma is difficult to diagnose and stage which might significantly affect the obtained results.

Another parameter subject to the analysis was the lymph node involvement status. The study revealed longer disease-free progression times in N(+) patients as compared to N0 patients; in addition, the analysis could not confirm that lymph node metastases contributed to longer survival. This might confirm errors in the accuracy of disease staging and/or presence of micrometastases in N0 patients. The role of lymphadenectomy in patients with advanced ovarian car-

cinoma is unclear. Some authors recommend intraoperational removal of pelvic and para-aortic lymph nodes, highlighting that lymph node metastases are observed in 50-70% of ovarian carcinoma patients. The researchers point out that this is the only way allowing for actual disease staging, as well as for the removal of bulky lymph nodes, thus significantly affecting the PFS, although having no effect on OS, as studies show. One should remember that besides lymph node metastases that accompany the disease spread, isolated lymph node metastases are also possible. In these cases, consideration of different therapeutic approaches, including radiotherapy, is recommended [13, 14].

An important result was the lack of the effect of the next-line chemotherapy on the OS as well as on the DFS, indicating therapeutic nihilism and the need to search for novel methods for the treatment of ovarian carcinoma. While radiotherapy of the entire abdominal cavity is controversial, the use of radiation in recurrent cancer and/or palliative treatment seems to be the main indication for the use of this technique in ovarian carcinoma patients [15, 16]. On the other hand, the only randomized trial that confirmed an increase in the OS and the PFS, as well as a reduction in the rate of recurrences, was the study conducted by Sorbe in which patients with advanced ovarian carcinoma after induction chemotherapy were subjected to radiotherapy of the entire abdominal cavity with higher doses targeted to the pelvic region [17].

The present study could not confirm the effect of radiotherapy on OS; however, it revealed good local control and positive impact on the DFS, particularly when the irradiation volume included para-aortic lymph nodes. The study results might have been affected by the total doses delivered during the treatments: some authors recommend doses as high as 45-60 Gy being delivered to bulky lymph node tumors in salvage therapy, while suggesting that even these doses might be insufficient [7, 10, 18, 19].

Blanchard *et al.* [13] analyzed a group of 640 ovarian carcinoma patients, 27 (4.2%) of whom developed isolated recurrences in lymph nodes. In 23 patients, the recurrence was correlated with an increase in the level of the Ca125 cancer marker. Most commonly, the recurrence was observed in retroperitoneal lymph nodes, followed by supraclavicular, mediastinal, mesenteric, and axillary lymph nodes. The treatment of recurrence episodes was varied. In 48% of cases, the disease recurrence was observed more than two years after cancer diagnosis. Mean OS was 68 months (12-210 months), while mean OS after lymph node recurrence was 26 months. Authors demonstrated no differences in two-year survival rates following a lymph node recurrence in the group of early recurrences (< 24 months) *vs.* the group of delayed recurrences (> 24 months) (59% and 47%, respectively). According to the authors, patients benefit most from radiotherapy in cases of isolated recurrence or recurrence in lymph nodes, particularly para-aortic lymph nodes [13].

Fujiwara *et al.* [20] assessed a group of isolated or multiple recurrences after disease spread was ruled out. The mean number of recurrences per patient was two (1-5), with a total of 44 recurrence foci in 20 patients. Patients were qualified to radiotherapy with total doses of 40-68 Gy administered in 1.6-2.0 Gy fractions. Tumor regression was observed immediately after treatment completion in 42 recurrences, with 39 recurrences responding within two to three months after the start of radiotherapy. The percentage of tumor regressions was higher in patients with lesions smaller than five cm and in cases when recurrences occurred in lymph nodes. However, according to the authors, disease progression outside radiation target volume occurred in most patients.

The study conducted by Tinger *et al.* [16] included 80 patients with ovarian carcinoma remission after at least one laparotomy and chemotherapy including one to 20 cycles of platinum-based agents. Some patients were subjected to radiotherapy targeting more than one site of recurrence and/or metastasis; in 64 patients, radiation was delivered to abdominal cavity, while 11 brain metastases and five metastases into other organs were also observed. Authors were able to achieve therapeutic response in 73% of patients, with complete resolution of symptoms, i.e. tumor mass and/or Ca 125 levels in 28% of patients. Partial response was observed in 45% of patients, while 11% of patients experienced progression during radiotherapy. Survival rates over periods of one, two, three, and five years after diagnosis were 89%, 73%, 42%, and 33%, respectively. Respective survival rates after completion of radiotherapy were 39%, 27%, 13%, and 10%, according to authors' estimates [16].

Many studies unambiguously point to the important role of radiotherapy in palliative treatment of ovarian carcinoma, particularly in metastases to the brain, bones, and lymph nodes [21]. Clinical symptoms may be related to recurrence within pelvis minor, as well as to metastases to the brain, lungs, and other locations. In their analysis of available literature, Delaney *et al.* [21] estimated that disease spread is observed in 38% of patients, with palliative radiotherapy indicated in 11% of patients. As shown by the studies, complete resolution of complaints is achieved after irradiation in 50-70% of patients [12, 15, 16, 20]. In about one-third of patients, the effect is maintained for about one year. The recommended total dose is about 30-45 Gy, depending on the general condition of the patient, cancer type and treatment history [3, 22].

Many authors recommend radiotherapy in palliative treatment of e.g. brain metastases, incidence of which has increased in recent years. Whole brain irradiation increases survival in ovarian carcinoma patients [3, 13].

Corn *et al.* [15] applied this treatment in 32 patients, demonstrating clinical response in 23 individuals. Of those, the response was maintained until death in 71% of cases.

The present study confirmed the importance of radiotherapy in palliative treatment, as patients subjected to this type of treatment had longer survival, although their DFS times

were shorter than in patients not subjected to radiotherapy due to distant metastases, obviously due to the stage of the disease. In addition, the study suggested that the control of 24-month survivals was better in the group of patients undergoing palliative radiotherapy, as compared to patients subjected to local radiotherapy. This indicates better radiotherapeutic control in the treatment of metastases as compared to local disease. The outcome might have been impacted by the fact that patients undergoing local treatment had history of multiple failures of other therapies (either surgical or systemic), where the treatment of metastases using irradiation was usually a primary treatment, commonly administered as a part of combination therapy.

The present study could not confirm the effect of brachytherapy on DFS. On the other hand, brachytherapy helped to achieve good local control, particularly within the first months after initiation of the treatment. The outcome might have been affected by the number of subjects, as clinical experience demonstrated an important role of brachytherapy in the treatment of ovarian carcinoma, particularly in advanced diseases including infiltration of uterine body and/or cervix and/or vaginal wall, as well as in helping to achieve good local control of the disease and/or antihemorrhagic effect [23].

The results presented herein as well as those reported by other authors suggest an important impact of adjuvant treatment in ovarian carcinoma patients, while indicating that radical surgery remains the imperative treatment goal as having an important effect on local control of the disease and increase in survival.

The results obtained in the present study should be subjected to further analyses in larger groups of patients; however, they may reflect inaccurate staging of the disease in patients in whom clinical presentation suggested a low stage and thus affected the extent of the procedure and impacted the overall survival and DFS.

Introduction of novel techniques of radiation planning facilitates irradiation of the largest possible volume within the abdominal cavity with the possibly lowest risk of early or delayed radiation-induced reactions in critical organs. The available techniques of intensity-modulated radiation therapy (IMRT), RapidArc, or tomotherapy may provide maximum protection of critical organs such as kidneys, liver, and intestines while ensuring precision and short treatment times when combined with image-guided radiation therapy (IGRT) [24-27].

It seems that the views on the role of radiotherapy in ovarian carcinoma treatment should be revised. The marginal use of radiotherapy should be extended by indications in the treatment of residual disease, post-surgery or post-chemotherapy residues, isolated inoperable metastases, recurrences, as well as in irradiation of the entire abdominal cavity in patients not qualified and/or resistant to chemotherapy or not consenting for other treatment methods. It should also be kept in mind that this treatment may be combined with intraoperative radiotherapy (IORT) in an increasing number of sites, providing an interesting alternative in the treatment of this disease [22, 26, 28].

Conclusion

In conclusion, the main limitation of the present study was the fact of its being a retrospective study in a small group of ovarian carcinoma patients. On the other hand, according to the Author's knowledge, this is one of few studies dealing with the role of radiotherapy in the treatment of ovarian cancer.

New prospective studies, designed to include the aspects of target volumes, total doses, and fraction doses, together with the use of state of the art planning techniques and therapeutic instrumentation are required. Other problems remaining to be solved are to determine the time point at which radiotherapy should be considered, whether the treatment should be administered in a standalone or combined fashion (together with chemotherapy and/or IORT and/or surgery), and what would be the appropriate order of therapeutic actions.

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Identification of potential miRNAs and candidate genes of cervical intraepithelial neoplasia by bioinformatic analysis

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Summary

Purpose: The objective of this study was to predict potential target genes and key miRNAs for cervical intraepithelial neoplasia (CIN) by bioinformatics analysis. *Materials and Methods:* The microarray data of GSE51993 were downloaded from Gene Expression Omnibus (GEO) database. Total 30 chips data from two platforms (each platform including eight CIN III samples data and seven normal cervix samples data) were used to identify the feature miRNAs and genes between CIN III and normal samples, respectively. Then the miRNA-mRNA regulatory network was constructed using Cytoscape software. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed for all target genes with the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool. Transcription factors (TFs) and cancer-related genes were analyzed. *Results:* Total 21 putative target miRNAs and 361 putative target mRNAs were gained. The miRNA-mRNA regulatory network results showed that miR-338-5p, miR-193a-5p, and miR-216b were top three hub nodes. GO terms significantly enriched were extracellular region ($p = 0.004191$) and embryonic skeletal system ($p = 0.004742$). No significantly enriched KEGG pathway term was found in this study. *PBX1* (pre-B-cell leukemia transcription factor 1) and *LAMC2* (laminin subunit gamma-2) were cancer-promoting genes and also, *PBX1* was TF. *Conclusions: PBX1* and *LAMC2* may be target genes for CIN. MiR-338, and miR-216 may be key miRNAs in CIN development.

Key words: Cervical intraepithelial neoplasia; Bioinformatics; miRNA-mRNA regulatory network.

Introduction

Cervical cancer, the malignant neoplasm of the cervix uteri, is the second most common cancer among women worldwide [1]. Cervical intraepithelial neoplasia (CIN) is considered a precursor of cervical cancer. Development of cervical cancer goes through several premalignant stages, from low-grade CIN (CIN I) through high-grade CIN (CIN II/ III) to cervical cancer [2]. These lesions have the ability to progress from hyperplasia to cervix, to preinvasive carcinoma, and ultimately to invasive carcinoma [3]. There is a general agreement that either ablation or excision of CIN-II, III could reduce the incidence and mortality caused by invasive cervical cancer in women with these lesions [4].

Numerous studies have been obtained in exploring the pathological mechanism underlying CIN development. MicroRNA (miRNA) has been found to play a key role in the CIN progression. MiRNAs are a class of 19 to 23 nucleotide single-stranded RNA molecules [5] and are epigenetic factors that regulate cell proliferation, tumor cell growth, cancer formation, and metastasis by regulating tumor suppressor genes or oncogenes [6]. Martinez *et al.* reported that miR-218 levels in patients with high-risk CIN were lower than those with low-risk CIN and therefore, downregulated miR-218 may be involved in the pathogenesis of cervical cancer [7]. In addition, tumor suppressor genes such as p16 and retinoblastoma proteins played roles in the neoplastic changes of CIN [8]. Previous studies showed that vascular endothelial growth factor (VEGF) expression was associated with the progression of CIN [9]. Using microarray gene expression data and bioinformatic analysis, Prashant *et al.* suggested that transcription factor (TF) family E2F played an important role in cervical carcinogenesis [10]. Moreover, apolipoprotein A1, a truncated form of transthyretin and a cleavage fragment of inter-α-trypsin inhibitor heavy chain H4 were identified to be potential biomarker [11]. Although many factors have been found in CIN development, the pathogenic mechanisms of CIN are still not clearly demonstrated, and it is lack of effective markers for CIN treatment.

In this study, the authors applied biological informatics methods to identify the feature genes and miRNAs between CIN III and normal samples. Additionally, the miRNA-mRNA regulatory network was constructed and the functional enrichment analysis was performed. The authors aimed to explore the molecular mechanism and discover the potential target genes and key miRNAs of CIN.

Materials and Methods

Microarray data

The data of GSE51993, which was deposited by Mo *et al*. on 2013, were downloaded from GEO (Gene Expression Omnibus) (http://www.ncbi.nlm.nih.gov/geo/) database based on the plat-

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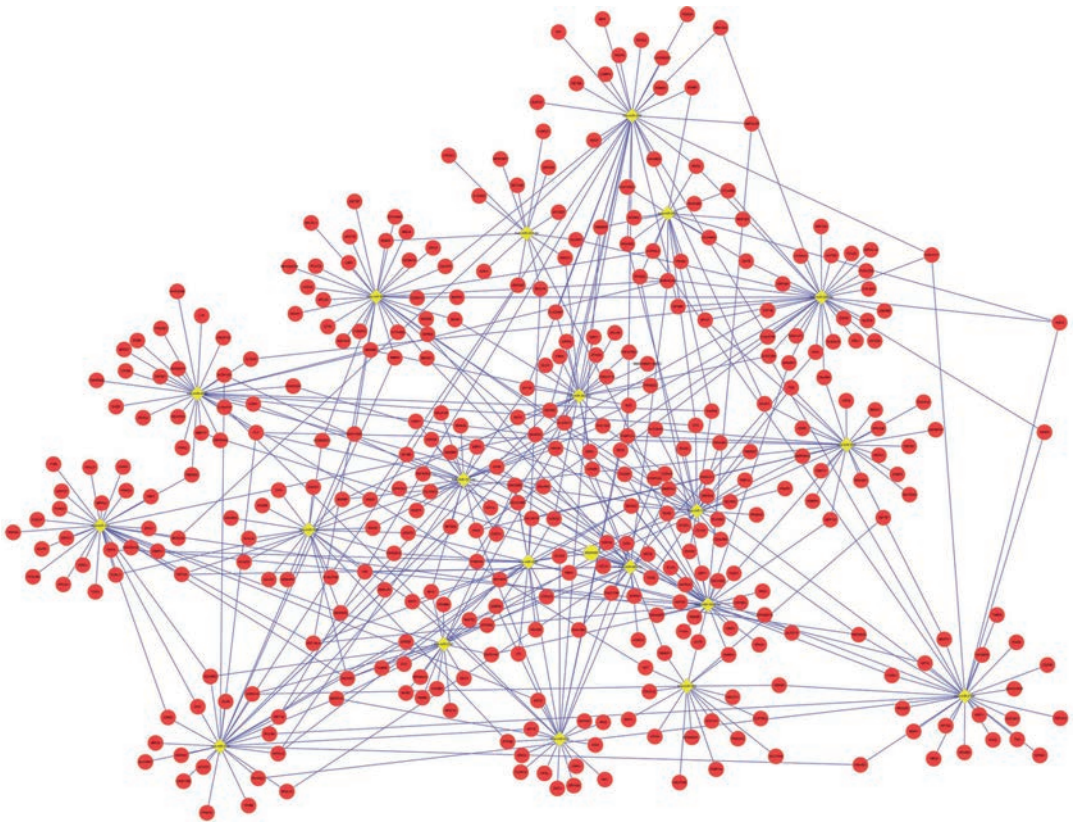

Figure 1. — Regulatory network of miRNAs and their target genes. Yellow nodes represent miRNAs; red nodes represent target genes related to miRNA.

form of GPL8179 (Human v2 microRNA expression beadchip) and GPL10558 (Human HT-12 V4.0 expression beadchip). Total 30 chips data from platforms of GPL8179 (15 chips data) and GPL10558 (15 chips data) were used for the development of the genome wide expression profiles of both miRNAs and mRNAs data, including eight CIN III samples data and seven normal cervix samples data, respectively. The data were normalized with robust multi-array average (RMA) [12] algorithm and subjected to logarithmic transformation.

Feature miRNAs and genes analysis

Probe sets were mapped to miRNAs and genes, respectively. Nonspecific probes were filtered. When multiple probe sets were mapped to the same miRNA or gene, the average expression value was calculated to represent the miRNA or gene expression level.

The feature miRNAs and genes between CIN samples and normal samples were analyzed. At first, interquartile range (IQR) [13] was used to filter miRNAs or genes based on gene expression levels distribution. All miRNAs or genes whose variability less than 1/5 overall IQR were eliminated. Then, ANOVA analysis was performed, and feature miRNAs or genes were filtered based on random forest [14].

TaLasso online analysis and miRNA-mRNA regulatory network

The TaLasso is both website (http://talasso.cnb.csic.es/) and the algorithm [15]. It has been tested with two datasets with matched miRNA and mRNA expression data.

The authors converted names of mRNAs and miRNAs which were screened from gene expression data into ensemble names. Then, data without ensemble name were deleted and the remaining data of mRNAs and miRNAs were put into TaLasso. The union of TarBase, miRecords, and miRWalk databases [15] were used for putative target genes. Cytoscape [16] is a software for visualizing complex networks and integrating networks between genes. With the application of Cytoscape software, the miRNA-mRNA regulatory network was constructed with these target genes.

Functional enrichment analysis

Gene Ontology (GO) database [17] is a collection of a large number of gene annotation terms. Kyoto Encyclopedia of Genes and Genomes (KEGG) knowledge database [18] is applied to identify the functional and metabolic pathway. Database for Annotation, Visualization and Integrated Discovery (DAVID) [19] is a gene functional enrichment analysis tool to understand the biological meaning for investigators. GO and KEGG pathway enrichment analysis were conducted with DAVID. A p-value < 0.05 was the cut-off criterion for the gene enrichment analysis.

Transcription factors and cancer related genes analysis

The Transcription Factors Database (TFD) [20] is a specialized database focusing on TFs and their properties. TFs, the target genes, were selected and identified from target genes based on TFD.

The tumor-associated gene (TAG) database [21] (http://www.binfo.ncku.edu.tw/TAG/) is designed to utilize information from well-characterized oncogenes and tumor suppressor genes to facilitate cancer research. Tumor suppressor genes (TSG) database [22] is a literature-based resource of tumor suppressor by integrating genomic data of mutations, gene expressions, regulations, methylations, and interactions. The cancer-promoting genes were extracted from TAG database, and tumor suppressor genes were extracted from TAG database and TSG database.

Table 1. — *Gene ontology analysis.*

GO category	GO ontology	GO term	Observed	*p*-value
GO:0005576	CC	Extracellular region	57	0.004191
GO:0048706	BP	Embryonic skeletal system development	7	0.004742
GO:0006959	BP	Humoral immune response	7	0.005376
GO:0048562	BP	Embryonic organ morphogenesis	9	0.005796
GO:0045087	BP	Innate immune response	9	0.0072

Note: CC: cellular component; BP: biological process.
Observed: number of the observed target genes in the category.

Results

Feature miRNAs and genes selection

Total 25 feature miRNAs were selected, including hsa-miR-338-5p, hsa-miR-193a-5p, hsa-miR-216b, hsa-miR-204, hsa-miR-21*, hsa-miR-887, hsa-miR-323-3p, hsa-miR-887, hsa-miR-1294, and so on.

Total 1,143 feature mRNAs were selected, including pre-B-cell leukemia transcription factor 1 (PBX1), laminin subunit gamma-2 (LAMC2), FBJ murine osteosarcoma viral oncogene homolog (FOS), chromodomain helicase DNA binding protein 1-like (CHD1L), chemokine (C-C motif) ligand 28 (CCL28), nicotinamide nucleotide transhydrogenase (NNT), nuclear receptor subfamily 0, group B, member 1 (NROB1), and so on.

TaLasso online analysis and miRNA-mRNA regulatory network

For putative target miRNAs and genes, 21 miRNAs and 361 mRNAs were gained. The miRNA-mRNA regulatory network was established with the 596 unique miRNA-target gene pairs (Figure 1). The degree of each miRNA in the network was then calculated and the top six were hsa-miR-338-5p (42), hsa-miR-193a-5p (38), hsa-miR-216b (37), hsa-miR-887 (35), hsa-miR-204 (35), and hsa-miR-21* (35).

Functional enrichment analysis

GO enrichment analysis were carried out for all target genes. The top five GO terms are shown in Table 1. The most significant term of cellular component (CC) was extracellular region (p = 0.004191), and that of biological process (BP) was embryonic skeletal system (p = 0.004742).

Transcription factors and cancer related genes analysis

In this study, 15 target genes, such as PBX1, homeobox D9 (HOXD9), homeobox B8 (HOXB8), and hepatocyte nuclear factor 4, gamma (HNF4G), were TFs. Five cancer-promoting genes including PBX1, LAMC2, FOS, CHD1L, and spalt-like transcription factor 4 (SALL4), as well as 14 tumor suppressor genes such as protein tyrosine phosphatase, non-receptor type 6 (PTPN6), microseminoprotein (MSMB), lactotransferrin (LTF), and histidine triad nucleotide binding protein 1 (HINT1) were detected.

Discussion

CIN is the leading cause of death among gynecological malignancies and represents the second-leading cause of cancer-related deaths in women worldwide [23]. Regardless of the fact that some genes have been reported in the progression of CIN or cervical cancer, there is a lack of detailed molecular pathogenesis mechanism. In this study, the authors identified the feature miRNAs and mRNAs between normal cervix samples and CIN III samples using bioinformatics analysis. Total 21 putative target miRNAs and 361 putative target genes were identified. TF analysis results showed that 15 TFs were associated with the regulation of CIN. Among these factors, *PBX1* and *LAMC2* were cancer-promoting genes. The miRNA-mRNA network was constructed, and miR-338-5p, miR-193a-5p, and miR-216b were hub nodes in this regulatory network.

PBX1 encodes a nuclear protein that belongs to the *PBX* homeobox family of transcriptional factors. *PBX* was a cofactor for HOX-class homeobox proteins [24]. Previous studies had shown that *HOX* and *PBX* genes were involved in oncogenic processes, such as chromatin binding, cell cycle control, proliferation, apoptosis, angiogenesis, and cell-cell communications [25-28]. Richard *et al.* reported that disrupting the interaction between HOX proteins and their co-factor PBX will retard tumour growth *in vivo* [24]. Thus, *HOX/PBX* interaction may be a potential target in cervical cancer therapy. In this study, *PBX1* was a feature gene and cancer-promoting gene, suggesting that it was possibly involved in oncogenic processes in the CIN. The miRNA-mRNA regulatory network results also showed that *PBX1* was regulated by miR-193a-5p which was the second hub node in the network. Previous study found that miR-193 regulated cell growth through the transforming growth factor-β (TGF-β) pathway by regulating Smad3 in glioma [29]. In addition, Chen *et al.* reported that miR-193b, another member of the miR-193 family, repressed cell proliferation and regulates cyclin D1 expression in melanoma [30]. Taking these factors into account, the present authors suggested that *PBX1* may play an important role in CIN development and miR-193a-5p targeting *PBX1* expression may be a critical event in CIN development. As well as *PBX1, LAMC2* is a cancer-promoting gene. It belongs to the laminin family, which is an epithelial basement membrane protein. It has been shown that LAMC2 was involved in a wide variety of biological processes including cell adhesion, differentiation, migration, and tumor invasion [31-33]. Immunohistochemical analysis revealed that

LAMC2 protein was highly expressed in cervix carcinomas [34] and was a marker to predict the risk of progression of CIN lesions [35]. Therefore, LAMC2 may be a key gene in CIN development.

Aside from miR-193a-5p, several other miRNAs such as miR-338 and miR-216b may be important in the development of CIN. It was reported that miR-338 suppressed the gastric cancer progression through PTEN-AKT signaling by targeting phosphatidylinositol-3,4,5-trisphosphat e-dependent Rac exchange factor 2 (P-REX2a) [36], as well as its role as a suppressor of the Smoothened-independent signaling pathways [37]. Furthermore, miR-216b was reported to suppress tumor growth and invasion by targeting KRAS (kirsten rat sarcoma viral oncogene homolog) in nasopharyngeal carcinoma [38]. Kim *et al.* showed that miR-216b promoted cellular senescence through the p53–$p21^{Cip1/WAF1}$ pathway in colon cancer [39]. In this study, miR-338 and miR-216b were hub nodes in miRNA-mRNA regulatory network. Thus, the present authors speculated that miR-338 and miR-216b may be key regulators in CIN development.

In order to predict potential target genes for CIN treatment, only CIN III and normal samples, not CIN II or CIN I, were analyzed. In conclusion, the genes of *PBX1* and *LAMC2* may play an important role in CIN development. These genes showed potential perspective in treatment of CIN. MiR-338 and miR-216 may be the key miRNAs in CIN development. They may be used to predict the risk of progression of CIN lesions. However, further experiments are still needed to confirm the present results.

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Comparison of the histopathological results of the endometrial thickness detected by transvaginal ultrasound of symptomatic and asymptomatic postmenopausal women

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Summary

Objective: The purpose of the study was to assess the reliability of transvaginal ultrasound (TVUSG) in endometrial pathologies by comparing the ultrasonographic and histopathologic findings in symptomatic and asymptomatic postmenopausal women. *Materials and Methods:* In this retrospective study the data of 129 postmenopausal women that underwent dilatation and curettage was reviewed by dividing them two groups as symptomatic and asymptomatic. Symptomatic group was divided into subgroups according to the value of endometrial thickness obtained by TVUSG. *Results:* Among all subjects the cancer rate was found statistically 3.043 times higher in patients with the endometrial thickness of 15 mm and greater and atrophic endometrium rate was 75% in patients with the endometrial thickness of less than five mm. Endometrial thickness was found significantly higher in cancer patients than the others ($p < 0.05$). Among the patients with endometrial thickness of 15 mm and greater, the cancer rate was found higher in symptomatic group than in the asymptomatic group. The cancer rate was found statistically higher in patients with bleeding compared to asymptomatic ones with the endometrial thickness between 5−14.99 mm ($p < 0.05$). Cancer was not detected in any of the symptomatic patients with the endometrial thickness of less than five mm. *Conclusion:* Postmenopausal patients with the symptom of bleeding should undergo detailed gynecological and ultrasonographic examination. The authors believe that this study may be a strong support to the success of TVUSG as a screening method in both symptomatic and asymptomatic postmenopausal women. Furthermore if the patient is symptomatic with a thick endometrium, to exclude the malignancy, endometrial biopsy must be performed.

Key words: Postmenopausal bleeding; Transvaginal ultrasonography; Endometrial thickness; Endometrium cancer; Menopause.

Introduction

Carcinoma of endometrium is the most common cancer of the female genital tract and 2-3% of all women seem to have endometrial cancer during their life span [1]. Its is diagnosed in 10% of patients with postmenopausal bleeding [2]. Because bleeding is the earliest symptom, all women with postmenopausal bleeding must be evaluated [3]. Transvaginal ultrasonography (TVUSG) is used as the first step because it is a safe, rapid, highly effective, and non-invasive method [4]. However the cut off level is still controversial [5].

The authors aimed to review the role of TVUSG to predict the risk of endometrial malignancy.

Materials and Methods

In this study, the authors retrospectively reviewed the data of 129 postmenopausal women, referred to the present hospital in a period of 27 months, between July 2010 and September 2012. The patients were between the ages of 46-83 years with the absence of menstruation for at least one year, provided that the amenorrhea was not explained by pregnancy, medication or disease.

A total of 129 women were included in the present study. Subjects were divided into two groups as 100 symptomatic patients (patients with the symptom of bleeding) and 29 patients with the endometrial thickness detected incidentally (asymptomatic patients). Patients with the symptom of bleeding were divided in three subgroups according to endometrial thickness calculated by using TVUSG (subgroup 1: < 5 mm, subgroup 2: ≥ 5 mm ≤ 15 mm, subgroup 3: ≥ 15 mm). Asymptomatic patients were divided into two subgroups (subgroup 1: ≥ 5 mm ≤ 15 mm, subgroup 2: ≥ 15 mm). Detailed physical and gynecological examinations of the patients were performed. Dilatation and curettage was performed in all subjects.

Peri- or premenopausal patients, the patients with the history of endometrial cancer or hyperplasia, and also the patients on hormonal replacement therapy or with the history of use were excluded. The authors also excluded the patients on tamoxifen therapy and the patients with the history of use.

TVUSG (with a high-frequency vaginal transducer of 6.5 MHz) was performed in all women. The cut off level was accepted as five mm. All women with postmenopausal bleeding, irrespective to their endometrial thickness, and asymptomatic women with thickened endometrium of five mm and more than five mm, were accepted as pathologic.

Histological materials were obtained by dilatation and curettage. Samples were kept in 10% formaldehyde solution and sent

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Table 1. — *Symptoms and findings of the patients and endometrial thickness.*

	N	%
Findings		
Vaginal bleeding	100	77.5
Thickened endometrium	29	22.5
Endometrial thickness		
< 5 mm	12	9.3
5-14.99 mm	84	65.1
≥ 15 mm	33	25.6

to pathology laboratories. They were assessed with an automatic tissue follower device for 13 hours. In this procedure, tissues were exposed to formaldehyde twice for 30 minutes each, to alcohol six times for 60 minutes, to xylene three times for 60 minutes, to paraffine once for 60 minutes, and twice for 80 minutes, respectively. After that sections with the thickness of two microns were dyed with Hematoxylin and Eosin. Sections were inspected by the same pathologist. The pathology results were reported as atrophic endometrium, irregular endometrial proliferation, polyp, simple hyperplasia without atypia, complex hyperplasia without atypia, complex hyperplasia with atypia, and cancers.

Statistical analysis

In this study SPSS (Statistical Package for Social Sciences) programme version 15.0 and GraphPad InStat demo version were used for the statistical analysis of the data. Beside the descriptive statistical methods, Chi-square test and Fischer exact test were used for the analyses of comparisons of the groups. Student's *t*-test and Mann Whitney U test were used for analyses of the comparison of two groups, and Kruskal Wallis test was used for the comparison of more than two groups. Dunn's test was used for the paired comparisons and Pearson correlation test was used for correlations. Confidence interval was 95% and significance level was accepted as $p < 0.05$.

Results

The mean age was 56.66 ± 8.68 years and the mean endometrial thickness was detected as 11.12 ± 6.02 (2.30-29.00) mm. The endometrium thickness was less than five mm in 9.3% (n=12), between five and 14.99 mm in 65.1% (n=84), and > 15 mm in 25.6% (n=33) of the patients (Table 1).

Among all subjects, atrophic endometrium was found in 42 patients (32.6%) and irregular endometrial proliferation was found in 20 patients (15.5%). In 34 patients (26.4%) there was a polyp, in seven patients (5.4%) simple hyperplasia without atypia, and in three (2.3%) complex hyperplasia without atypia. Only one patient (0.8%) had complex hyperplasia with atypia. Twenty-two patients (17.1%) were reported as cancer in the present study (Table 2).

The endometrial thickness was less than five mm in 12 patients, between 5−14.99 mm in 62 patients, 15 mm and greater in 26 patients in the group with the symptom of bleeding. The endometrial thickness was between 5−14.99 mm in 22 patients and 15 mm and greater in seven patients in asymptomatic group. When all the patients were divided into three groups according to their endometrial thickness (< 5 mm, between 5−14.99 mm, and ≥ 15 mm) the cancer rate was found statistically higher in patients with the endometrial thickness of 15 mm and greater, compared to the other two groups ($p < 0.05$). The cancer rate was 3.043 times more (95% Cİ: 1, 1.68−7.930) in patients with an endometrial thickness of

Table 2. — *Pathology results.*

	n	%
Pathology result		
Atrophic endometrium	42	32.6
Irregular endometrial proliferation	20	15.5
Polyp	34	26.4
Simple hyperplasia without atypia	7	5.4
Complex hyperplasia without atypia	3	2.3
Complex hyperplasia with atypia	1	0.8
Endometrioid adenocarcinoma	16	12.4
Serous papillary carcinoma of endometrium	1	0.8
Mixed type: 70% squamous cell ca. 30% adenocarcinoma	1	0.8
Adenocarcinoma with papillary differentiation	1	0.8
Squamous cell carcinoma in situ of cervix	1	0.8
Clear cell carcinoma	1	0.8
Breast cancer with metastasis	1	0.8
Cancer		
Undiagnosed	107	82.9
Diagnosed	22	17.1

Table 3. — *Clinical features according to endometrial thickness.*

	< 5 mm		5−14.99 mm		≥ 15 mm	
	n	%	n	%	n	%
Findings						
Bleeding	12	100.0	62	73.8	26	78.8
Thickened endometrium	0	0.0	22	26.2	7	21.2
Pathology result						
Atrophic endometrium	9	75.0	31	36.9	2	6.1
Irregular endometrial proliferation	2	16.7	14	16.7	4	12.1
Polyp	1	8.3	22	26.2	11	33.3
Simple hyperplasia without atypia	0	0.0	3	3.6	4	12.1
Complex hyperplasia without atypia	0	0.0	2	2.4	1	3.0
Complex hyperplasia with atypia	0	0.0	0	0.0	1	3.0
Cancer	0	0.0	12	14.3	10	30.3
Cancer						
Undiagnosed	12	100.0	72	85.7	23	69.7
Diagnosed	0	0.0	12	14.3	10	30.3

Table 4. — *The relationship between bleeding and cancer according to endometrial thickness.*

	No cancer		Cancer present	
	n	%	n	%
< 5 mm				
without bleeding	0	0.0	0	0.0
with bleeding	12	100.0	0	0.0
5−14.99 mm				
without bleeding	22	100.0	0	0.0
with bleeding	50	80.6	12	19.4
≥ 15 mm				
without bleeding	6	85.7	1	14.3
with bleeding	17	65.4	9	34.6

15 mm and greater, than in ones with the thickness less than 15 mm (Table 3).

The authors examined the relation of cancer and bleeding by dividing the patients into three groups according to the endometrial thickness (< 5 mm, between 5-14,99 mm, and ≥ 15 mm). They compared the symptomatic patients with with asymptomatic ones with the same endometrial thickness. The cancer rate was found to be higher in patients with bleeding compared to asymptomatic ones with the endometrial thickness of ≥ 15 mm. Cancer diagnosis rate was found statistically higher in bleeding patients compared to asymptomatic ones with an endometrial thickness between 5-14.99 mm ($p < 0.05$) (Table 4). Cancer was not detected in the symptomatic patients with an endometrial thickness of < 5 mm. Although endometrial thickness was found to be lower in patients with symptom of bleeding compared to asymptomatic patients, it was not found to be statistically significant ($p > 0.05$).

Discussion

The authors assessed the reliability of TVUSG in endometrial pathologies by comparing the ultrasonographic and histopathologic findings in symptomatic and asymptomatic postmenopausal women in this study. According to the results they found that the cancer rate was statistically 3.043 times (95% CI: 1.168−7.930) higher in patients with an endometrial thickness of ≥ 15mm. There were no cancer detected in the group of patients with the endometrial thickness of ≤ 5 mm.

Postmenopausal bleeding is an important sign of both cervical and endometrial pathologies which should be kept in mind in differential diagnosis. Cervical cancer screening methods widely used all over the world however still do not include a widely accepted screening method for the endometrial pathologies. The high incidence of endometrial cancer in postmenopausal patients with bleeding requires a simple diagnostic method with a high accuracy rate. In postmenopausal women endometrium becomes atrophic and the thickness of endometrium is measured as 2.3 ± 1.8 mm as a result of hormonal changes [6]. Hence endometrial thickness is also a suspicious sign in postmenopausal women. TVUSG is used as the first step method of examination because it is cheap, reliable, and non-invasive [4].

There are several studies in the literature similar to the present one. Gull *et al.* followed 339 women who had postmenopausal bleeding for ten years and 39 of them had endometrial cancer and five had hyperplasia with atypia. They found the ratio of relative risk of endometrial cancer in women who were referred with postmenopausal bleeding as 64. None of the endometrial cancer cases were missed when the endometrial thickness cut-off value was taken as four mm. They concluded that TVUSG was an excellent tool for the determination of whether endometrial biopsy was necessary [7]. They explained these high figures of endometrial cancer during the observational period of 10 years, by the fact that some of the initial biopsy results were actually false negative which made the incidence too high.

In the present study, 34.6% of the symptomatic patients with an endometrial thickness > 15 mm were diagnosed as cancer. According to the present study it can be stated that one-third of symptomatic patients with endometrial thickness > 15 mm was detected as cancer. This high ratio of cancer in the present clinic depends on the fact that the hospital is tertiary centre and these patients with the higher probability of cancer might have been referred to the present clinic.

It is proper to accept the cut-off value of endometrial thickness as five mm [4]. Haller *et al.* measured the double layer endometrial thickness of 81 patients by TVUSG one day prior to hysteroscopy and curettage. They showed that TVUSG detected 46 of 48 pathologic conditions including all cases of endometrial carcinoma when the cut-off value was five mm (sensitivity 95.8%, specificity 45.5%) [8]. The present authors also consider cut-off value as five mm as in the mentioned studies. Atrophic endometrium was found in 75% of the patients with an endometrial thickness of less than five mm in the present study and none of them were cancer. In a multi-centric study in which 1,168 patients were followed in eight different clinics of four Nordic countries, none of the patients with an endometrial thickness of less than five mm were detected as cancer. They also suggested that curettage should not be performed in these patients [4].

When the present authors divided the patients into three groups according to endometrial thickness, they found a higher cancer rate in symptomatic (bleeding) group than the asymptomatic ones who had ≥ 15 mm and statistically higher rate in the group of 5−14.99 mm endometrial thickness ($p < 0.05$). This result shows that cancer rate is much higher in the patients with bleeding symptom than in the asymptomatic ones with the same endometrial thickness. Thus, bleeding should seriously alert the physician.

Goldstein suggested that postmenopausal bleeding was cancer until proven otherwise. He pointed out that, the risk of malignancy of a thin endometrium was so small like one in 917. The rate of asymptomatic endometrial thickening was at least 10% in postmenopausal women and polyps were the reason in most of the cases. He also mentioned that the risk of malignancy in polyps was 0.1%. Complication incidence in such postmenopausal women was not negligible (1.3−3.6%). Hence intervention in such women without any high risk status was not logical [9]. Unlike his suggestion, in the present study the authors found cancer rate to be statistically 3.043 times (95% CI: 1.168−7.930) higher in patients with the endometrial thickness of ≥ 15 mm. Depending on this result the present authors would like to point out that a thick endometrium of a postmenopausal woman may be a potential sign of endometrial cancer and should be taken seriously; if the endometrial thickness is found to be five mm or greater, biopsy should be performed even if the patient is asymptomatic.

As a result, the postmenopausal patients with symptom of bleeding should undergo detailed gynecological examination and TVUSG should be performed. Furthermore if the patient is symptomatic with a thick endometrium, endometrial biopsy must be performed in order to exclude malignancy. TVUSG is an effective and non-invasive method for the diagnosis and follow up of the patients who are at high risk of endometrial cancer. The present authors think that this study may be further strong support to the success of TVUSG as a screening method in both symptomatic and asymptomatic postmenopausal women.

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Health information quality on the internet in gynecological oncology: a multilingual evaluation

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Summary

Background: Oncological internet information quality is considered variable, but no comprehensive analysis of gynecological malignancies exists. The present authors' objectives were to compare the quality of common malignancy websites and to assess for language or disease differences; and secondly, to perform a quality comparison between medical and layperson terminology. *Materials and Methods:* World Health Organization (WHO) Health on the Net (HON) principles may be applied to websites using an automated toolbar function. Using a search engine (www.Google.com) 8,400 websites were assessed using keywords 'endometrial, 'uterine', 'cervical', 'ovarian', 'vaginal', 'vulvar', plus 'cancer', in English, French, German, and Spanish; repeated for alternate terms e.g. 'cervix', 'womb'. *Results:* Searches for "vaginal' 'uterine', 'cervical', and 'endometrial' each returned millions of websites. The total percentage of all assessed HON-accredited sites was notably low across all search terms (median 15%; range 3-19%). Significant differences by malignancy type ($p < 0.0001$), language ($p < 0.0001$), and tertiles (thirds) of the first 150 websites returned ($p < 0.0001$). French language had most accredited websites. Using alternate terms demonstrated significant differences ($p < 0.001$) in accredited websites for most gynecological cancers. *Conclusions:* Internet data on gynecological malignancies is overwhelming. Further, a lack of validation of the majority of gynecological oncologic sites should be appreciated with discrepancies in quality and number of websites across diseases, languages, and also between medical and layperson terms. Physicians should encourage and more importantly their professional bodies should participate in the development of informative, ethical, and reliable health websites on the internet and direct patients to them.

Key words: Gynecology; Neoplasms; Internet; Patient education; Women.

Introduction

Patients appear to trust information on the Internet as much as from other media [1]. Thus it is unsurprising that the Internet and Social Media have become an accessible source of health-related information for patients and other interested parties [2]. Almost 80% of Internet users, which comprised almost 60% of all American adults, use the Internet to seek medical information [3, 4]. This highlights the importance of assessing the quality and validity of such information. The importance of the internet within obstetrics and gynecology was predicted in 1997 [5] and is likely to continue [6]. However, studies of the quality of oncological health information published on the Internet have found it restricted, variable, and overwhelming - patients may be faced with millions of results for a simple term search [7-9].

Patients and doctors are confronted with a confusing array of educational, promotional, and even complication-oriented websites regarding diseases and treatment options in oncology [10,11]. However, the content of internet resources and video forums is largely unregulated without the rigor that is applied to scientific publications [12]. There is a danger of misinformation leading to a negative impact on patient's understanding, expectations, choice of therapy, choice of care, and ultimately quality of life [8, 13, 14]. Oncology poses other interesting challenges for patients. For example, if the cancer is endometrial, they may search for 'endometrial cancer' but equally may choose alternate terms such as 'uterine', 'endometrial adenocarcinoma' or even 'womb cancer'. The choice of descriptive terms for a type of cancer has been found to make a difference with the quality of information presented by an internet search [9]. Furthermore, information quality differs based on language [7-9, 15-17] which is of importance in multicultural societies and non-English speaking countries.

Clinicians are also placed in difficult situations when confronted with internet resources and need tools to identify quality information for themselves and to direct patients [8, 9]. Several systems have been developed to help identify quality and reliable health information [7, 8, 16]. The Health on the Net (HON) Foundation is a not-for-profit accreditation body supported by the World Health Organization (WHO) that is multilingual and has the goal of accrediting health websites using key principles of authority, complementarity,

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Table 1. — *Results of the total websites returned for each term and also the percentage of HON accredited sites (poHONA). Also, the poHONA according to for websites in tertiles (first, second, and third 50) for each search returned is also indicated. Total websites and percentage of HON-accredited sites by treatment options.*

Terminology	Term searched	Total websites returned[a]	HON accredited (600 per term)[b] HONCODE +	HONCODE -	Total	PoHONA (%)	*p* value
Endometrial cancer	Endometrial cancer	1,320,00	111	489	600	19	
	Endometrial adenocarcinoma	321,000	86	514	600	14	
	Womb cancer	1,150,000	87	513	600	15	
	Uterine cancer	2,400,000	92	508	600	15	
TOTAL (median*, sum^)		1,235,000*	376^	2024^	2400^	15*	0.173
Cervical Cancer	Cervical cancer	16,939,000	112	488	600	19	
	Cancer cervix	12,939,000	113	487	600	19	
	Cervical intraepithelial neoplasia	570,440	55	545	600	9	
TOTAL (median*, sum^)		12,939,000*	280^	1520^	1800^	19*	< 0.001
Ovarian cancer	Ovarian cancer	15,100,000	80	519	600	13	
	Ovary cancer	2,010,000	94	506	600	16	
	Ovarian carcinoma	1,250,000	85	515	600	14	
TOTAL (median*, sum^)		2,010,000	259^	1540^	1800^	14*	0.501
Vaginal Cancer	Vaginal cancer	1,660,000	103	497	600	17	
	Vaginal SCC**	410,000	84	516	600	14	
	Vulvar cancer	441,000	98	502	600	16	
	Vulvar SCC**	215,000	82	518	600	14	
Total (median*, sum^)		425,500*	367^	2033^	2400^	15*	0.15
Grand total (median*, sum^)		1,622,500	1066^	5964^	8400^	15* (3-19)	

a – total web sites returned = total of four languages: English, French, German, and Spanish; b – Total of 600 per term = four languages x 150 web sites searched; **SCC = squamous cell carcinoma.

confidentiality, attribution, justifiability, transparency of authorship, sponsorship, and advertising [8, 18].

The present authors' objective was to compare the quality of gynecological oncology websites based on the HON principles and to assess for language or disease differences across Western languages being English, French, German, and Spanish. A further aim was to perform a quality comparison between search results when medical rather than layperson terminology or even alternate terms are used for a particular malignancy.

Materials and Methods

Internet searching for websites

The authors' methodology has been previously described and utilised in previous publications [8, 9]. Using the Google search engine (www.Google.com), in February 2014, the authors performed internet searches for 15 terms associated with gynecological oncology (e.g. endometrial and uterine cancer, Table 1) and assessed 8,400 websites. English and equivalent terms in French, German, and Spanish (translated from English through use of medical translation services and confirmed by laypersons and doctors having the non-English primary language as their primary language for term accuracy) were utilised.

Internet searching for accredited websites

Based on the observation that patients rarely access more than the first page of search results [19], the first 150 websites yielded by each search were then identified and sequentially screened for quality as defined by the HON Foundation. This was done by applying HON principles through the HONcode toolbar function [18] for use on any personal computer and automatically activates or "lights-up" toolbar if a website is accredited by the HON foundation). The HON function has been used and assessed in several studies and was thus deemed to be a valid and high calibre tool [8, 20].

Analysis of accredited websites likelihood of being viewed

A secondary analysis of the first 150 websites encountered for each tumor type was undertaken as previously described [8, 16, 21]. Firstly, all returned websites for each cancer were divided into tertiles (first, middle, and last 50). The proportion of accredited sites in each organ and language was then analysed and compared using the Chi-square test. The purpose of this analysis was to determine where accredited websites were appearing preferentially i.e. in the pages least likely (last 50) versus the most likely to be viewed (first 50).

Quality control

For quality control, an English search ('cervical carcinoma'), had non-accredited sites within the first 150 discovered websites manually evaluated using the HON criteria to determine their HON status to ascertain if they fulfilled the criteria despite not being "officially" accredited.

Logistic regression examining variables associated with HON status

This was conducted using the three major variables of search term, language, and tertiles of the first 150 returned. The referent groups for each variable were the English version and the first 50 websites respectively as these had the highest percentage and/or number of HON accredited websites.

Analysis of website sponsors

For all organ groups, an analysis was undertaken from English language websites to determine the website sponsors (individual or

Table 2. — *Differences in HON accreditation of websites by term and language. PoHONA = percentage of HON accredited sites. The p values refer to comparison within each terminology/treatment group.*

Terminology/ Treatment	English			French			German			Spanish			*p* value
	HON CODE+	HON CODE-	Po HONA	HON CODE+	HON CODE-	Po HONA	HON CODE+	HON CODE-	Po HONA	HON CODE+	HON CODE-	Po HONA	
Uterine cancer													< 0.0001
Uterine cancer	24	126	19	33	117	28	20	130	15	15	135	11	
Endometrial cancer	33	117	28	39	111	35	13	137	9	26	124	21	
Endometrial adenocarcinoma	26	124	21	33	117	28	6	144	4	21	129	16	
Womb cancer	19	131	15	33	117	28	20	130	15	15	135	11	
TOTAL	102	498	20	138	462	30	59	541	11	77	523	15	
Cervical carcinoma													< 0.0001
Cervical cancer	37	113	33	42	108	39	10	140	7	23	127	18	
Cancer cervix	38	112	34	42	108	39	10	140	7	23	127	18	
Cervical intraepithelial neoplasia	25	125	20	15	135	11	4	146	3	11	139	8	
TOTAL	100	350	29	99	351	28	24	426	6	57	393	15	
Ovarian carcinoma													< 0.0001
Ovarian cancer	23	126	18	31	119	26	8	142	6	18	132	14	
Ovary cancer	37	113	33	31	119	26	8	142	6	18	132	14	
Ovarian carcinoma	22	128	17	29	121	24	10	140	7	24	126	19	
TOTAL	82	367	22	91	359	25	26	424	6	60	390	15	
Vaginal squamous cell carcinoma													< 0.0001
Vaginal cancer	24	126	19	23	127	18	22	128	17	34	116	29	
Vaginal SCC*	26	124	21	23	127	18	18	132	14	17	133	13	
Vulvar cancer	35	115	30	20	130	15	13	137	9	30	120	25	
Vulvar SCC*	16	134	12	26	124	21	24	126	19	16	134	12	
Total	101	499	20	92	508	18	77	523	15	97	503	19	
Grand total (median*)	385	1714	20.5*	420	1680	26*	186	1914	8*	291	1809	15*	

organization responsible for the website) and each was categorized according to prior studies of quality of websites on the internet [7, 8]. In summary, the sites were deemed sponsored by (1) lawyers; (2) non-profit organizations; (3) government organizations /educational institutions; (4) commercial; (5) surgeons/ physicians (and their professional organizations); (6) other health professionals; or (7) other. Sponsorship was determined independently by two examiners firstly by web page retrieved; if sponsorship was not obviously apparent, the website was explored until sponsorship could be determined. The concept of sponsorship is not to be confused with the Google terminology of "sponsored links" either highlighting pages at the start of retrieved search or appearing on the side of the page under a banner. As in prior analysis, such pages were not included throughout the entirety of this study [8].

Statistical analysis

Comparisons of proportions across types of cancer and language were performed using the Chi-square test (or Fisher's exact tests when cell counts were less than 5). All statistical tests were two-sided and significance was defined as $p < 0.05$. Odds ratios and 95% confidence intervals (CI) were also calculated from the logistic regression analysis. Analyses were performed using SAS 9.1.

Results

Internet search results for accredited websites

The total number of websites for each disease term was variable (Table 1). The terms 'vaginal' 'uterine', 'cervical', and 'endometrial' each returned millions of websites. 'Cervical cancer' the most around 17 million, closely followed by 'ovarian cancer' at 15 million, while 'vulvar squamous cancer' had the fewest - around 200,000.

Including all languages, the total percentage of all assessed HON-accredited (poHONA) sites was notably low across all search terms (median 15%; range 3-19%, Table 1). Most search terms did record at least 10% poHONA but this is still very modest. The quality control analysis of English language for 'cervical carcinoma' revealed further 4% of websites that would likely be of a standard equivalent to HONcode accredited sites but have yet to apply for accreditation.

In regards to linguistic differences (Table 2), French (median 26%; range 11-35%), had the greatest percentage of HON-accredited sites across all disease search terms, followed by English (21%; 12-34%), Spanish (15%; 8-29%) and German (9%; 3-19%).

When analysing by tertiles to determine where HON-accredited sites were more likely to appear, it appears that HON-accreditation was significantly more common in the sites that appear in first tertile (Figure 1).

Finally the ORs were calculated demonstrating significant differences with search terms, language or between groups (Table 3). Indeed it appeared an Internet search was almost equally likely to be accredited provided the key term for that malignancy was used. However, the first tertile was

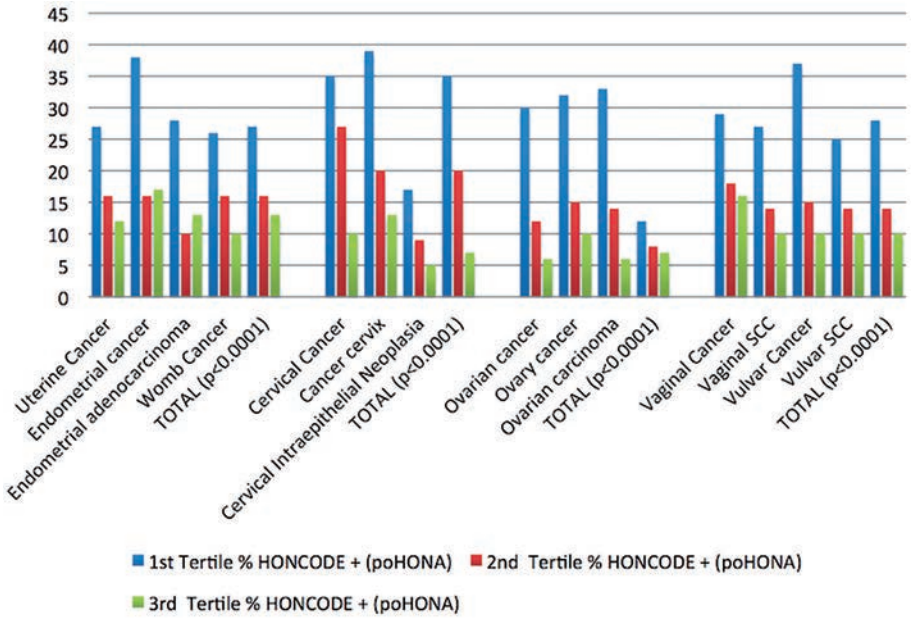

Figure 1. — Graph of the results of the percentage of HON accredited sites (poHONA) by organ group. Also, the percentage of HONCODE + accredited websites (poHONA) according to tertiles (first, second, and third fifty sites) for each search returned is also indicated. Statistical analysis of differences within each organ malignancy for tertiles is displayed in the total column.

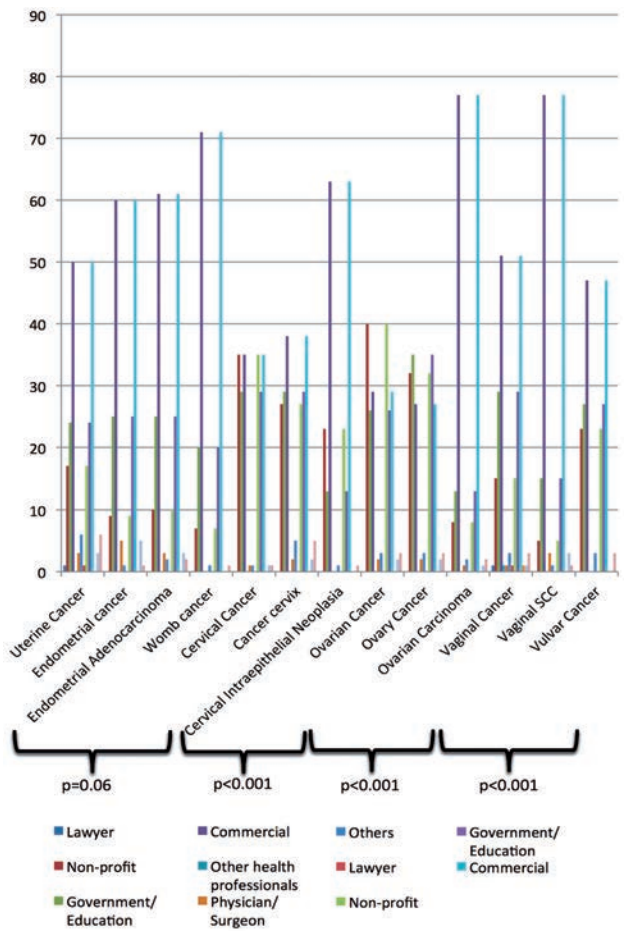

Figure 2. — Graph of the results of the analysis of website sponsors by organ malignancy (or alternate term) across the English language sites only. Statistical analysis for differences within each malignancy group is highlighted in the total columns.

Table 3. — *Results of the logistic regression analysis comparing across gynecological oncology terminology, likelihood of an accredited website based on first, second, and third 50 websites returned and by language. Referents were chosen based on the term cervical carcinoma and its alternate terms being the standard; the first tertile returned because of this having the greatest percentage of HON accredited websites and English as the most common language.*

Effect on HONCODE status	Odds ratio	95% confidence limits
Category		
Cervical carcinoma	1.00 (referent)	
Ovarian carcinoma	0.909	0.754 - 1.096
Uterine carcinoma	1.009	0.849 - 1.198
Vaginal/vulval carcinoma	0.963	0.782 - 1.185
*Websites^*		
1st tertile (0-50)	1.00 (referent)	
2nd tertile (51-100)	0.501	0.435 - 0.577
3rd tertile (101-150)	0.349	0.299 - 0.408
Language		
English	1.00 (referent)	
French	1.117	0.956 - 1.306
German	0.427	0.354 - 0.516
Spanish	0.710	0.600 - 0.840

^Of the first 150 websites examined, the first third or 50 (five pages) were reference group compared to second third and last third.

more likely to return an accredited site over the second (OR 0.50) and third (0.31). Comparing to English language, French sites were slightly more likely to return an accredited site (OR 1.12) while least likely in German (OR 0.43) with Spanish between (OR 0.71).

Analysis of website sponsors

The sponsor analysis of the 150 Websites in English language revealed (Figure 2) that the most commonly encountered sponsors were commercial sites (51%) followed by Government organisations or educational institutions (29%) and non-profit organisations (23%). Other sponsors (15%), other health professionals (4%), surgeons/physicians (4%) sponsored far less sites and lawyer–sponsored sites were rarely if ever encountered.

Discussion

The Internet is a tool that assists health providers and patients daily. Data exists that women and their families are seeking information specifically on the Internet and via social media [22, 23]. Interestingly they may even be asking questions on the internet that they find too private to even discuss with their doctors [24, 25]. It has been recognised that the internet has been utilised to improve colorectal cancer screening and this could be translated into ovarian or cervical cancer screening [26]. This adds to the logic that as physicians we need to direct patients to reliable information.

General gynecological related resources have demonstrated a pattern of poor quality [27, 28]. Website content for gynecological malignancies has never been specifically studied until now. The quality may appear low in this study but is in fact is similar to that found in surgical and urologic oncology where routinely around 20% or fewer websites are HON accredited [8, 9]. Gynecological malignancies are somewhat similar to breast (23%) [9] but performing similar to prostate cancer (16%) [8] on available data. Language differences exist regarding Website quality [7, 8, 15]. In the present study, French-language searches overall had more Website listings and ultimately had more HON-accredited sites as compared to English, German, and Spanish. At best, just over a quarter of French were HON-accredited and at worst, under one-tenth of German websites were HON-accredited. It is interesting that French again outperformed English as was the case with multiple common non-gynecological and non-urologic malignancies in a previous study [9]. English only outperformed French and others languages with urologic malignancies [8]. Combining this with the differences between malignancies suggests differing regional interest and resource allocation to specific malignancies based not just on incidence but other factors.

The incidence and prevalence of cancers as well as their "public profile" are all likely to be factors influencing the number of websites available to consumers. When searching the different gynecological malignancies, 'layperson terms' generally performed worse than the correct medical terminology. This was also demonstrated in other organ oncological website searches attesting to the importance of gynecological oncology [29]. Teaching patients and their support groups how to search the Internet is important and adhering to medical terminology is an important finding for health providers and patients. Advising them of how to access the free HONcode toolbar to "screen" websites of higher quality would also be prudent.

The Internet although a wonderful "free" and "open access" resource is increasingly being subjected to commercialisation [16]. This is often at the expense of considered, well-balanced opinion. Website sponsorships which are often not obviously disclosed as well as the creation of hidden metrics for search retrieval means there may be biases in what information is returned. This study highlights the paucity of good quality comprehensive, multilingual information on available on the Internet and confirms commercial sponsors to be the greatest providers of such information.

There are a number of limitations of the study. The Internet is dynamic with Websites constantly being developed and uploaded. Thus search results may vary depending on time and location. Furthermore other search engines are available apart from 'Google', hence future analyses could investigate if alternate internet filter systems would significantly alter the quality of websites retrieved.

Internet data on gynecological malignancies is overwhelming. A lack of validation of the majority of gynecological oncologic sites should be appreciated with discrepancies in quality and number of websites across diseases, languages and also between medical and 'layperson' terms. Interestingly, the quality found is similar for oncological internet searches for other disease groups. Physicians should encourage and participate in the development of informative, ethical, and reliable health websites on the internet and direct patients to them. Importantly, professional bodies need to act swiftly to become "trusted sources" that physicians and patients may rely upon in an increasingly "digital world'.

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Application of Cervista® human papilloma virus high-risk test in cervical cancer screening of Xinjiang Uyghur women

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Summary

Objective: This study aims to evaluate the diagnostic value of the Cervista® human papilloma virus (HPV) high-risk (HR) test in cervical cancer and precancerous lesion screening in Xinjiang Uyghur women. *Materials and Methods:* Three hundred and seventy three Uyghur women from Bachu County underwent the Cervista® HPV HR test, ThinPrep cytologic test, and cervical biopsy under a colposcope. Then the relationship between the infection rate of high-risk human papilloma virus (HR-HPV), the cytological results, and the histological results was analyzed. *Results:* With increasing cytological and pathological classification, the HR-HPV infection rate also increased and reached 100% in patients with cervical cancer. The highest proportion of the pathologically positive group consisted of patients with group A9 HPV infection as compared to patients infected with group A5/A6 or A7 HPV. *Conclusion:* HR-HPV is closely correlated with cervical cancer and precancerous lesions. Group A9 HPV has a high predictive value in cervical disease screening in Uyghur women. When cytological examination shows atypical squamous cells of undetermined significance (ASCUS), the Cervista® HPV HR test can provide a sensitive differentiation method.

Key words: Cervical cancer; Cervista HPV HR test; ThinPrep cytologic test.

Introduction

Nowadays, cervical cancer is the only cancer that has the potential to be destroyed. Persistent infection of high-risk human papilloma virus (HR-HPV) has been recognized as a major risk factor for cervical cancer [1]. The clear pathogenic factors of cervical cancer and the slow development of the disease offer a valuable opportunity for mass screening and treatment. A large number of clinical practices show that cervical precancerous lesions can be found by screening and that they can be blocked before the occurrence of cervical cancer. The ThinPrep cytologic test (TCT) combined with HPV detection is recommended as the first screening method. However, cytological examinations should be performed by experimental cytologists and include continuous quality control, which is difficult in regions without adequate medical resources. HPV detection for cervical cancer and precancerous lesions has high sensitivity and good repeatability, stability, and objectivity, which is suitable for cervical cancer screening in regions without adequate medical resources. The effectiveness of cervical cancer screening depends on the high sensitivity of HPV DNA detection methods.

For women, in terms of incidence, cervical cancer ranks second among malignant tumors worldwide. Particularly in developing countries and regions, it ranks first [2, 3]. The incidence of cervical cancer and death in Xinjiang is the highest in Xinjiang in China. In Xinjiang, especially in Southern Xinjiang, cervical cancer is the leading cause of death in Uyghur women. Its prevalence and mortality are much higher in Uyghur women than in women of other ethnicities living in the same region, and the onset age is also lower than that in other ethnic populations. Among all the minorities in China, Uyghur women have the highest mortality due to cervical cancer [4]. Thus, an economical and effective screening method for cervical cancer is of great significance for the rural areas of the Xinjiang Uyghur Autonomous Region. The cervical cancer screening program in Bachu County was supported by the National Natural Science Foundation of China and the Chinese Cancer Foundation. This program was in cooperation with the Maternal and Child Health Hospital of Bachu County. In this program, a series of commonly used cervical cancer screening technologies were systemically evaluated. The Cervista® HPV HR test was used in this study for screening HR-HPV in Uyghur women in the Bachu County of Xinjiang in order to explore its diagnostic value in detecting cervical cancer and precancerous lesions in Uyghur women. Additionally, the test could provide a scientific basis for the differential diagnosis of patients with a cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS).

Materials and Methods

Subjects

Among the 5045 Uyghur women who attended the screening program in Bachu County, any woman who tested positive for the care HPV test, TCT, or acetate/iodine staining was recalled for colposcope examination and cervical biopsy of suspicious lesions.

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Among the patients who underwent a colposcope examination, 373 patients were randomly selected to undergo the Cervista® HPV HR DNA test. The patients were 20 to 65 years old, with a mean age of 39.25 ± 8.65 years. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Xinjiang Medical University. Written informed consent was obtained from all participants.

Diagnosis using the TCT

Diagnosis using TCT results was based on the new Bethesda system classification standards recommended by the National Cancer Institute in 2001. According to the severity of cell atypia, the specimens could be classified as normal cells, inflammation, or ASCUS, including the atypical squamous cells of highly squamous intraepithelial lesions (ASCH), low-grade squamous intraepithelial lesions (LSIL), highly squamous intraepithelial lesions (HSIL), and squamous cell carcinoma (SCC).

Cervista® HPV HR test

The 14 kinds of HR-HPV that could be detected by the Cervista® HPV HR test, could be divided into the following three groups: group A5/A6 (HPV51, HPV56, and HPV66), group A7 (HPV18, HPV39, HPV45, HPV59, and HPV68), and group A9 (HPV16, HPV31, HPV33, HPV35, HPV52, and HPV58). DNA extraction and the Cervista® HPV HR test were carried out according to the manufacturer's instructions.

Pathological grading

Cervical biopsies could be diagnosed histologically as chronic cervicitis, cervical intraepithelial neoplasia grade I (CIN I), CIN grade II (CIN II), CIN grade III (CIN III), and cervical SCC (cervical cancer I and infiltrating carcinoma).

Statistical analysis

All results were analyzed using SPSS 18.0 software. The positive rates of HR-HPV in the different grades of cervical lesions were compared using the Chi-square and rank sum tests. A value of $\alpha = 0.05$ was used as the test standard, and $p < 0.05$ was considered statistically significant.

Results

Cytological diagnosis by TCT

Among the 373 samples, there were 265 normal specimens (71.0%), 25 cases of ASCUS (6.7%), ten cases of ASCH (2.7%), 52 cases of LSIL (13.9%), and 21 cases of HSIL (5.6%). A total of 108 patients (29%) displayed ASCUS or further cytological changes.

Relationship between HR-HPV infection and the cytological grades

Among the 265 cytologically normal samples, there were 210 (79.2%) HR-HPV-positive cases. Among the 25 cases of ASCUS, 21 (84%) were HR-HPV positive, while among the patients with ASCH, nine (90%) were HR-HPV positive. In patients with LSIL and HSIL, the HR-HPV infection rates were 100%. The HR-HPV infection rate in the samples with different cytological grades showed a statistically significant difference ($c^2 = 20.958$, $p < 0.05$; Table 1). The HR-HPV infection rate increased with the cytological grades.

Table 1. — *HR HPV infection rates in different cytological grades of samples.*

Cytological diagnosis	Cases (n)	HR HPV-positive (cases)	Positive rate of HR HPV
Normal	265	210	79.2%
ASCUS	25	21	84.0%
ASCH	10	9	90.0%
HSIL	21	21	100.0%
LSIL	52	52	100.0%
Total	373	315	84.5%

Table 2. — *The infection rates of group A9, A5/A6, and A7 HR HPV in the different grades of cytological abnormalities.*

Groups	A5/6 (cases)	A7 (cases)	A9 (cases)	Total (cases)
ASCH	1	1	8	10
ASCUS	8	1	16	25
HSIL	7	2	19	28
LSIL	12	4	42	58
Normal	74	55	130	259
Total	102	63	215	380

Relationship between cytological abnormalities and the infection rates of different HR-HPV groups

According to the Cervista® HPV HR test results, there were 102 cases of group A5/A6 HPV infection (26.8%), 63 cases of group A7 HPV infection (16.6%), and 215 cases of group A9 HPV infection (56.6%). The infection rate of group A9 HPV was prominently higher than that of the other two groups, including single infection and coinfection with two or three groups. The infection rates of group A9, A5/A6, and A7 HR-HPV in the ASCUS, LSIL, and HSIL cases showed a statistically significant difference ($c^2 = 19.544$, $p < 19.544$; Table 2).

Association of HR-HPV infection rates with pathological grades

Based on the histological results, 373 patients were classified into the chronic cervicitis (n = 247), CIN I (n = 68), CIN II (n = 26), CIN III (n = 22), and cervical cancer (n = 10) groups. The positive rate of HR-HPV in the chronic cervicitis group was 78.1% (193/247), significantly lower than the rates in the CIN I, CIN II, CIN III, and cervical cancer groups, respectively (95.6% [65/68], 100.0% [26/26], 100% [22/22], and 100% [10/10], respectively; [$p < 0.05$]; Table 3). The HPV infection rate increased with the increase in the pathological grade. Among the 315 HR-HPV-positive cases, chronic cervicitis, CIN I, CIN II, CIN III, and cervical cancer accounted for 61.3% (193/315), 20.6% (65/315), 8.3% (26/315), 7.0% (22/315), and 3.2% (10/315) of cases, respectively. The HR-HPV-positive cases included single infection and mixed infection. The infection rates for group A5/A6, group A7, and group A9 HR-HPV were 26.8%, 16.6%, and 26.8%, respec-

Table 3. — *HR-HPV infection in patients with different pathologic grades.*

Histologic grades	Number of cases	HR-HPV positive	Positive rate
Cervical cancer	10	10	100.0%
CIN III	22	22	100.0%
CIN II	26	26	100.0%
CIN I	68	65	95.6%
Chronic cervicitis	247	193	78.1%
Total	373	315	84.5%

Table 4. — *The distribution of HR-HPV positive cases in different pathologic grades.*

Groups	A5/6 (cases)	A7 (cases)	A9 (cases)	HPV-positive (cases)	HPV-negative (cases)
Chronic cervicitis	73	45	118	236	54
CIN I	18	13	46	77	3
CIN II	5	3	22	30	0
CIN III	5	2	20	27	0
Cervical cancer	1	0	9	10	0
Total	102	63	215	380	57

tively. In the chronic cervicitis, CIN I, CIN II, CIN III, and cervical cancer groups, the infection rate of group A9 HR-HPV was markedly higher than the infection rates of the other groups ($c^2 = 16.288$, $p < 0.05$; Table 4).

Discussion

HPV infection is highly associated with the occurrence and development of CIN and cervical cancer. It has been proven that the HPV infection rate is almost 100% in the cervical tissues of cervical cancer patients. Currently, there are many methods for HPV detection, among which hybrid capture 2 (HC2) HPV DNA detection was the first method to be approved by the Food and Drug Administration. The HC2 HPV DNA test can detect 13 types of HR-HPV. However, it has some limitations, such as false negatives due to a low level of virus infection or uncorrected sampling, potential cross-contamination, cross-reaction of the HR type probe with the low-risk type probe, high cost, complex operation, large time requirements, and so on. The Cervista® HPV HR DNA test is a new HPV detection method that has also been approved by the Food and Drug Administration recently. It uses Invader chemistry, a signal amplification method for detecting special nucleic acid sequences, rather than the polymerase chain reaction, so that the amplification of erroneous signals can be avoided. Two synchronous isothermal reactions run at the same time, which can improve the specificity and anti-interference capability. Cervista® is the only method with an internal control, which can reduce false-negative results caused by insufficient sample DNA, and has no cross-reaction with low-risk HPV types, which reduces the chance of false positives. Cervista® detects the L1 and E6/7 regions of HPV simultaneously, significantly improving the specificity, and avoiding the false-negative results of methods that only detect the L1 region. It has been reported that there is no significant difference between the HPV-positive rates in the HC2 and Cervista® HPV HR assays, when a population with no intraepithelial lesions or malignant lesions is screened [5-8]. The Cervista® HPV HR test was approved for clinical use by China's State Food and Drug Administration in November 2011. Researchers compared the Cervista® HPV HR test with gene sequencing for HPV detection and got good consistency [9].

Worldwide, the distribution and genotype of HPV differs from region to region and nationality to nationality. Currently, HPV16 is the main type of HPV found in cervical tissues worldwide, followed by HPV18. HPV16/18, HPV31, 33, 35, 45, 52, and 58 are the other subtypes found in cervical cancer [10, 11]. Chinese women have much higher HPV16 infection than in other countries, accounting for 79.6% of all cervical cancers. However, the positive rate of other important HPV types differs in different regions [12, 13]. The distribution of HPV infection also differs according to gender [14]. It has been reported that HPV16 is the most common type of infection in Tibetan and Yao women [15, 16], but the other types have a different distribution in Tibetan and Yao women. For instance, HPV33 and HPV58 rank second and third, respectively, in Tibetan women, but in Yao women, HPV52 and HPV58 are the second and third most common infections, respectively. Previous findings regarding cervical cancer showed that HPV16 is the most common infection type in Uyghur women, followed by HPV58 [17]. This study showed that the HR-HPV infection rate increased with the increasing grade of cytological diagnosis and reached 100% in cervical cancer, further confirming the important role of HR-HPV in the development of cervical cancer. The present findings also showed that the infection rate of group A9 HPV was the highest, followed by group A5/A6. The present results were consistent with the results reported [18-22], suggesting that group A9 HR-HPV has high pathogenicity and a high risk of causing cervical cancer. The high infection rate of group A9 HPV in the cervical lesions of Uyghur women may be related to the high infection rates of HPV16 and HPV58 in Uyghur women, which are both in group A9. Thus, attention should be paid to the Uyghur women infected with HR-HPV, especially with the types in group A9, and early intervention should be taken.

ASCUS presents with more obvious cytomorphological changes than reactive changes, but does not reach the level of squamous intraepithelial neoplasia. It can be a benign lesion with active proliferation or a potential malignant lesion [23]. The treatment of ASCUS is always difficult using the standard treatment of cervical lesions, resulting in excessive or insufficient treatment or delayed diagnosis and treatment. Einstein *et al.* pointed out that in women with cytomorphological ASCUS [24], HR-HPV has a sensitivity of 92.8% and negative predictive value of 99.1% for CIN2+ and a sensitiv-

ity of 100% and positive predictive value of 100% for CIN3+. However, its specificity for CIN2 and CIN3 were only 44.2% and 43%, respectively [24]. The Cervista® HPV HR assay showed that group A9 HPV has high pathogenicity, which can be used as a sensitive differential diagnosis method for the cervical cytomorphological examination. For patients with cytomorphological ASCUS, the Cervista® HPV HR test should be performed. If the specimen is group A9-positive, a colposcopy and cervical biopsy should be performed immediately in order to make a clear diagnosis and provide effective treatment in time. Patients without HR-HPV infection can have regular cytological examinations. It does not only save medical resources and reduce the unnecessary cervical injury caused by colposcopy examination, but also reduces the economic and psychological burden of the patients.

Conclusion

In this study, Cervista® HR HPV DNA test is firstly used to screen cervical lesions in Xinjiang Uyghur Women. The authors aimed to discuss predictive value and found A9 group has high level of pathogenicity, which provides a sensitive shunt method for cervical cytology examination. Further study will focus on consistency of Cervista® HR HPV DNA and HC2, and further validate accuracy of the method.

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Biweekly administration of docetaxel and carboplatin for advanced or recurrent endometrial and ovarian carcinomas

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Summary

Objective: To examine efficacy and safety of biweekly administration of docetaxel and carboplatin for advanced or recurrent endometrial and ovarian carcinomas. *Material and Methods*: The recommended doses were determined in the phase I study. In the phase II feasibility study, the primary end-point was safety, and the secondary end-point was response rate and progression-free survival (PFS). *Results*: The recommended doses of docetaxel and carboplatin were determined to be 45 mg/m^2 and AUC 3.0, respectively, in phase I study. In phase II feasibility study, no treatment-related death was observed. Most non-hematotoxicity cases were mild or moderate. Grade 4 neutropenia was confirmed in 13 patients (31.0%), whereas all cases showed tolerability with 2.6 days delay of anticancer drugs administration in both groups. Response rate was 55.0% in the ovarian carcinoma group, and average PFS was 8.7 months. In the endometrial carcinoma group, response rate was 50.0% and average PFS was 32.0 months. *Conclusion*: The present results showed that biweekly administration of docetaxel and carboplatin for advanced and recurrent endometrial and ovarian carcinomas results in acceptable side effects, response rate, and PFS.

Key words: Biweekly administration; Docetaxel; Carboplatin; Endometrial carcinoma; Ovarian carcinoma.

Introduction

Currently, taxane is widely used for treatment of a variety of malignant tumors throughout the world. The standard therapy for ovarian cancer is combined administration of paclitaxel with carboplatin every three weeks [1]. The response rate of paclitaxel and carboplatin administrated was 63-87% [2-4], and a high efficacy of the therapy has been shown in advanced or recurrent endometrial carcinoma. However, neurotoxicity, which is one of the side effects caused by paclitaxel, sometimes becomes severe and interferes with the treatment [5, 6].

Docetaxel exerts its anticancer effect by binding to microtubules and inhibiting depolymerization of the microtubules in the same manner as paclitaxel. In a comparative study of combined administration of paclitaxel with carboplatin and combined administration of docetaxel with carboplatin every three weeks as the first-line chemotherapy for patients with ovarian carcinoma, no significant differences were found in the progression-free survival (PFS) and response rate (58.7% *vs.* 59.5%) [7]. With regards to side effects of docetaxel and carboplatin administrated, however, notable strong myelosuppression and grade 3-4 neutropenia occurred (94% *vs.* 84%) compared to combined administration of paclitaxel with carboplatin. On the other hand, occurrence rates of neurotoxicity were 45% and 78% for neurosensory ($p < 0.001$) and 9% and 16% for neuromotor ($p < 0.001$), and were significantly mild [7].

Meanwhile, mitigation of side effects is expected with weekly administration of taxane and platinum, compared to a concomitant use of taxane and platinum with administration every three weeks, which is the standard administration. A number of clinical trials have been conducted to examine the efficacy of the weekly administration [8-11]. However, the weekly administration requires frequent office visits, which is inconvenience for patients and expected to increase healthcare costs. Results of some studies indicate that concomitant use of docetaxel and carboplatin by biweekly administration show favorable tolerability in patients with lung cancer [12, 13]. However, no study has been conducted to assess the efficacy and safety of biweekly administration of docetaxel and carboplatin for ovarian carcinoma and endometrial carcinoma, which would be very meaningful. Thus the present authors designed a phase I/II trial to examine efficacy and safety of biweekly administration of docetaxel and carboplatin for advanced or recurrent endometrial and ovarian carcinomas.

Materials and Methods

Patients

Twenty patients with ovarian carcinoma and 22 patients with endometrial carcinoma who gave their written agreement between April 2003 and October 2006 were included in this study. The median age of the patients with ovarian carcinoma and patients with endometrial carcinoma was 55.8 (35-69) years and 63.2 (49-74)

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years, respectively. ECOG Performance Status was 0 for seven patients, 1 for nine patients, and 2 for four4 patients in the ovarian carcinoma group and 0 for 11 patients, 1 for six patients, and 2 for five patients in the endometrial carcinoma group. According to the classification of International Federation of Gynecology and Obstetrics, two patients (10.0%) were classified as Stage IC, 16 patients (80.0%) as Stage IIIC, and two patients (10.0%) as Stage IV for advanced stages at the initial diagnosis in the ovarian carcinoma group, and eight patients (36.4%) were classified as Stage IB, five patients (22.7%) as Stage II, three patients (13.6%) as Stage IIIA, one patient (4.5%) as Stage IIIB, two patients (9.1%) as Stage IIIC1, and three patients (13.6%) as Stage IVB for advanced stages at the initial diagnosis in the endometrial carcinoma group. All of 20 patients with ovarian carcinoma were recurrent cases, four patients were determined as recurrent because the CA125 value became two times higher than the upper limit of the reference value, the others had measurable disease. Nineteen of 22 patients with endometrial carcinoma were recurrent cases, and all cases, included three advanced cases (Stage IVB), had measurable disease.

Methods

To examine efficacy and safety of biweekly administration of docetaxel with carboplatin therapy, recommended dose were determined in the phase I study; in the phase II feasibility study, the primary end-point was safety, and the secondary end-point was response rate and PFS. Of recurrent and advanced epithelial ovarian carcinoma and recurrent and advanced endometrial carcinoma cases, patients who were 20 years and older and below 75 years of age, whose ECOG Performance Status was 0-2, who maintained major organ functions, and who gave written agreement were included in the study. Intravenous drip infusion was conducted biweekly, and dose-limiting toxicity (DLT) was grade 4 hematotoxicity and Grade 3 non-hematotoxicity. As premedication, eight mg of dexamethasone and antiemetic agent (5HT3 antagonist) were dissolved into 100 ml of saline and administered by intravenous drip infusion 30 minutes before the docetaxel administration. Docetaxel was dissolved into 250 ml of 5% glucose solution or saline, and administered by intravenous drip infusion over 60 minutes. Carboplatin was dissolved into 100 ml of saline, and administered by intravenous drip infusion over 60 minutes.

Initial dose was 40 mg/m^2 for docetaxel and AUC 3.0 for carboplatin (level 1). Docetaxel and carboplatin were 35 mg/m^2 and AUC 3.0 for level 0, 45 mg/m^2 and AUC 3.0 for level 2, and 50 mg/m^2 and AUC 3.0 for level 3, respectively. Table 1 shows dose-escalation scheme. First, one cycle of level 1 administration was given to three patients, and the presence or absence of adverse drug reaction in each patient at each administration level was observed. The adverse reaction was evaluated according to the National Cancer Institute - Common Toxicity Criteria (NCI-CTC Version 3.0) [14]. Anticancer drugs at a dose determined at level 1 were administered in three patients, and the incidence of side effects was observed. The level was increased one level when no DLT occurred, and maximum-tolerated dose (MTD) was determined as the dose one level lower than the level at which DLT occurred in all three patients. When DLT occurred only in one or two of three patients, the same amount was administered in three new patients again, and the level was further increased if DLT occurred in two patients or less in six patients. If DLT occurred in at least three patients, MTD was determined as the dose one level lower.

Phase II feasibility study was conducted based on the recommended dose obtained in phase I. Toxicity was evaluated in all patients who received the treatment at every cycle. NCI-CTC Version 3.0 was used for the evaluation [14]. Response evaluation was conducted as follows for patients with a lesion available for two-dimensional measurement. The evaluation was conducted two times with at least four week intervals. Complete response (CR) was defined as CR of all measurable lesions and evaluable lesions determined by two separately conducted determinations. Partial response (PR) was defined as at least 50% decrease in the sum of the product of the vertical diameter of an evaluable lesion. Progressive disease (PD) was defined as a 25% or greater increase in the sum of the product of the vertical diameter of an evaluable lesion or appearance of new lesions. NE was defined as changes was not evaluable. Stable disease (SD) was defined as changes that do neither correspond to CR, PR, PD nor not evaluable (NE).

When no evaluable pathological changes were observed and recurrence was determined because of increase of CA125 value over the upper limit of the reference value or at least two times increase of the nadir level, efficacy was determined according to the CA125 criteria [15].

Table 1. — *Dose-escalation scheme.*

	DOC (mg/m2)	CBDCA AUC
Level 0	35	3.0
Level 1	40	3.0
Level 2	45	3.0
Level 3	50	3.0

Table 2. — *Hematologic toxicity of ovarian cancer group.*

Hematologic	G3	G4	Grade 4 (%)
Neutropenia	3	6	6 (30.0)
Febrile neutropenia	0	0	0
Anemia	3	1	1 (5.0)
Thrombocytopenia	2	0	0

Table 3. — *Hematologic toxicity of endometrial cancer group*

Hematologic	G3	G4	Grade 4 (%)
Neutropenia	4	7	7 (31.8)
Febrile neutropenia	0	0	0
Anemia	3	0	0
Thrombocytopenia	3	0	0

Results

Determination of recommended dose

No DLT was observed in three cases at level 1 or level 2. The recommended doses of docetaxel and carboplatin were determined to be 45 mg/m^2 and AUC 3.0 at level 2, respectively, in phase I study, because DLT was observed in all three cases at level 3.

Toxicity

No treatment-related death was observed. Hematotoxicity results of ovarian carcinoma group and endometrial carcinoma group are summarized in Tables 2 and 3, respectively.

Table 4. — *Non-hematologic toxicity of ovarian cancer group*

Non-hematologic	G1	G2	G3	G4	Grade 3-4 (%)
Anorexia	1	2	0	0	0 (0)
Nausea/vomiting	3	4	0	0	0 (0)
Fatigue	4	2	0	0	0 (0)
Diarrhea	2	1	0	0	0 (0)
Alopecia	3	1	0	0	0 (0)
Neuropathy	2	1	0	0	0 (0)
Dysgeusia	4	0	0	0	0 (0)
Myalgia	1	0	0	0	0 (0)
ALT/AST	2	1	0	0	0 (0)
Nail change	4	1	0	0	0 (0)
Stomatitis	1	1	0	0	0 (0)
Allergic reaction	2	0	0	0	0 (0)

Table 5. — *Non-hematologic toxicity of endometrial cancer group*

Non-hematologic	G1	G2	G3	G4	Grade 3-4 (%)
Anorexia	2	2	0	0	0 (0)
Nausea/vomiting	4	4	0	0	0 (0)
Fatigue	5	2	0	0	0 (0)
Diarrhea	1	1	1	0	1 (4.5)
Alopecia	4	1	0	0	0 (0)
Neuropathy	1	1	0	0	0 (0)
Dysgeusia	3	0	0	0	0 (0)
Myalgia	2	0	0	0	0 (0)
ALT/AST	3	1	0	0	0 (0)
Nail change	4	0	0	0	0 (0)
Stomatitis	1	2	0	0	0 (0)
Allergic reaction	1	0	0	1	1 (4.5)

Anemia caused as a side effect of the treatment was confirmed in three patients (15.0%) for grade 3 and one patient (5.0%) for grade 4 in the ovarian carcinoma group. Anemia in either patient was improved by blood transfusion, but anticancer drug administration was delayed for three days and dose was reduced to the amount of level 1 for the latter patient. On the other hand, in the endometrial carcinoma group, anemia was confirmed in three patients (15.0%) for grade 3. Neutropenia was confirmed in seven patients (16.7%) for grade 3 and 13 patients (31.0%) for grade 4; however, administration of anticancer drugs was conducted after an average of 2.6 days of postponement because of administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF). No patient developed febrile neutropenia in both groups. Non-hematotoxicity results of ovarian carcinoma group and endometrial carcinoma group are summarized in Tables 4 and 5, respectively. Most non-hematotoxicity cases were mild or moderate, and were transient with the exception of one case of endometrial carcinoma in which the treatment was discontinued because of anaphylactic shock during the third course of the treatment and one case of frequent diarrhea. In addition, the color of the nails of nine patients changed into dark brown (grade 1), and one patient experienced deformation and loss of nails (grade 2). Neuropathy was observed in three patients at grade 1 and in two patients at grade 2.

Response and survival

On average, administration was conducted 8.3 times (ovarian carcinoma group, 7.8 (1-15) times; endometrial carcinoma group, 8.6 (4-12) times). Response rate was 55.0% in the ovarian carcinoma group (CR: eight cases, PR: three cases, SD: one case, PD: three cases, NE: one case), and average PFS was 8.7 months. In the endometrial carcinoma group, response rate was 50.0% (CR: five cases, PR: six cases, SD: one case, PD: three cases, NE: three cases) and average PFS was 32.0 months. Table 6 shows the response rate.

Table 6. — *Response rate.*

	CR	PR	SD	PD	NE	Response rate (%)
Ovarian cancer (n=20)	8	3	1	3	1	55.0
Endometrial cancer (n=22)	5	6	1	3	3	50.0

Discussion

Taxane anticancer agents have previously indicated efficacy against ovarian and endometrial carcinomas. It is reported that the response rate of paclitaxel alone for advanced or recurrent endometrial carcinoma is 27-36% [16, 17], and 21-34% for docetaxel [18-20]. Doxorubicin plus cisplatin therapy (AP) and cyclophosphamide, doxorubicin, and cisplatin therapy (CAP) have been used for endometrial carcinomas since the 1980s [21, 22]. It was shown that AP therapy is superior to doxorubicin alone for advanced or recurrent endometrial carcinoma by two randomized controlled trials of European Organization for Research and Treatment of Cancer and Gynecologic Oncology Group [23, 24].

Recently, TAP therapy (concomitant use of paclitaxel 160 mg/m^2, doxorubicin 45 mg/m^2, and cisplatin 50 mg/m^2, G-CFS), which is a three-drug combination-chemotherapy including paclitaxel in addition to AP therapy, was examined in a randomized controlled trial (GOG 177). Response rate, PFS and OS of the TAP therapy were all significantly superior, but toxicity of TAP therapy, especially neuropathy, was more severe than that of AP therapy [25]. Based on these results, AP therapy is used as the first-line therapy for endometrial carcinoma in general community hospitals. However, doxorubicin has cardiotoxicity. It is reported that administration of doxorubicin at 550 mg/m^2 or higher caused significantly higher rate of congested heart failure [26]. Therefore, administration of doxorubicin at 550 mg/m^2 or higher was associated with a great risk even if the patient was determined as doxorubicin sensitive. Combination therapies of plat-

inum-based chemotherapy and paclitaxel are also used as front-line treatment for endometrial carcinoma in many facilities; however, only few therapies are effective for patients with these therapies-resistance.

It is hoped that docetaxel will be an alternative anticancer drug to paclitaxel. This is because docetaxel has 2.5 times higher effect on microtubules than paclitaxel [27]. Of 15 patients with paclitaxel-resistance ovarian carcinoma, CR was observed in five cases (33.3%), and PR in three cases (20.0%) in the present study. In addition, patients that previously received paclitaxel were excluded in a study previously reported on advanced or recurrent endometrial carcinoma [18-20]. In the present study, CR and PR respectively were observed in two patients (28.6%), respectively that previously received paclitaxel in endometrial carcinoma. Therefore, the present authors consider that a better effect can be expected from concomitant use of docetaxel and carboplatin.

In the present study, concomitant use of docetaxel and carboplatin by biweekly administration for advanced or recurrent ovarian carcinoma obtained 55.0% of CR and PR. There is a phase II trial that administered docetaxel (75 mg/m^2) and carboplatin (AUC: 5) every three weeks for recurrent ovarian, peritoneal, and tubal carcinoma as docetaxel plus carboplatin therapy [28]. Subjects of this trial were 25 patients with platinum sensitive recurrent ovarian carcinoma who experienced carboplatin alone or combination therapy of carboplatin and other anticancer drug as 9 first line chemotherapy; a high response rate of 72% was reported. Of these 25 cases, 21 cases received a combination therapy of paclitaxel and carboplatin; thus, it is considered that docetaxel will be an effective anticancer drug for recurrent ovarian carcinoma after therapy of paclitaxel and carboplatin.

Docetaxel has toxicity characteristics different from paclitaxel, although both are taxane agents [27]. Neutropenia is the most common toxicity of docetaxel. In the present study, grade 4 neutropenia was found in nine cases (45.0%) of ovarian carcinomas, and ten cases (45.5%) of endometrial carcinomas. Strauss *et al.* reported that grade 3 or worse neutropenia was observed in 60% of patients in their study that administered docetaxel (75 mg/m^2) and carboplatin (AUC: 5) every three weeks in recurrent ovarian carcinomas [28]. Moreover, grade 3 or worse neutropenia were observed in 94% of patients in every three-week administration of docetaxel (60 mg/m^2) plus carboplatin (AUC: 5), as well as febrile neutropenia, which required postponement of treatment for at least seven days, was observed in 14% of patients in a phase III trial that compared docetaxel plus carboplatin and paclitaxel and carboplatin as a first-line chemotherapy for ovarian carcinomas [7]. Also, in a comparison study of three arms including every three-week administration of docetaxel plus cisplatin, docetaxel plus carboplatin, and paclitaxel plus carboplatin for advanced recurrent ovarian carcinomas, grade 3 or worse neutropenia was observed in 90% of patients and febrile neutropenia was observed 6.7% of patients in the docetaxel plus carboplatin group [29]. For neurotoxicity, grade 3 motor neuropathy (6.7%) and grade 3 sensory neuropathy (1.3%) were observed in paclitaxel plus carboplatin group. On the other hand, neuropathy of grade 3 or worse was not observed in the docetaxel plus carboplatin group [29]. No neuropathy case of grade 3 or worse was found in the present study.

The present results showed that biweekly administration of docetaxel or carboplatin for advanced and recurrent endometrial and ovarian carcinomas results in acceptable side effects, favorable response rate, and PFS. It is suggested that biweekly administration of docetaxel and carboplatin maybe a front-line chemotherapy for advanced or recurrent endometrial and ovarian carcinomas. However, a further randomized phase III study would be required to evaluate risks and benefits of biweekly administration of docetaxel and carboplatin.

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Allelic polymorphism in codon 72 of p53 gene: prognosis value, survival rates, and their association with breast cancer

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Summary

Purpose of investigation: There are controversial findings to establish relationship between genotype polymorphism of codon 72 of *P53* gene, its prognosis value, and survival rate of the patients in breast cancer. For the first time this study has shown such relationship in Sabzevar, Iran. *Materials and Methods:* A descriptive analytical case–control study was conducted on 160 people (80 patients and 80 controls). DNA was extracted and codon 72 of the p53 gene was amplified. The genotype of the p53 gene was determined by electrophoresis, samples were sequenced, and all patients were followed up for 30 months. *Results:* The frequency of heterozygote arginine/proline was 49 (30.6%) and 51(31.9%) in the patients and controls, respectively. Homozygote arginine/arginine had frequency of 29 (18.1%) in the patients while it was 15 (9.4%) in controls. Homozygote of proline/proline was two (1.3%) in the patients and 14 (8.8%) in controls. The sequencing results were consistent to PCR and electrophoresis results. *Conclusions:* This is the first study in the region which shows relationship between genotype polymorphism, survival rate, and its prognosis value in breast cancer. The authors showed that homozygote proline/proline in controls was significantly higher compared with that in the patients. They may therefore, conclude that detection of allelic polymorphisms of codon 72 of the p53 gene including arginine/arginine could be a risk factor predisposition for breast cancer and valuable tool for determining prognosis, progress, and treatment purposes.

Key words: Breast cancer; Allelic polymorphism; Codon 72.

Introduction

Breast cancer is one of the most leading causes of deaths in the world and the second one in western countries [1]. The prevalence of the disease is also increasing in Asian populations [2]. In 90% metastasized patients, the resistance to routine chemotherapies has been reported [3]. Different epigenetic or genetic factors [4] have been assumed to be involved in the process of the disease including inhibition in apoptosis and impairment in the repair of DNAs [5, 6]. The p53 gene, located on the short arm of chromosome 17 with 11 exons has been identified for its role to inhibit impairments in cycle and growth of cells [7] in apoptosis, transcription, and senescence [8 ,9]. Several studies including the authors' previous study [10] showed that polymorphism and some mutations in the p53 gene play roles in changes which lead to malignancy in colorectal [11] breast, lung, and bladder cancers [12, 13]. It has been suggested that polymorphism in the codon 72 of the exon 4 of the p53 gene has link to breast cancer. It has two allelic forms which lead to produce arginine (CGC) and proline (CCC) in p53 protein respectively [14]. It has been also reported that the risk of breast cancer has link with homozygocity of CGC genotype, arginine/arginine phenotype [15]. In contrast, some studies concluded that there is no such association between breast cancer and the polymorphism in the codon 72 of the p53 gene [16-18]. However, an association between homozygocity of proline (CCC) and the prognosis of breast cancer has been reported in Finland by Tommiska *et al.* [19].

Controversial results from different parts of the world may be due to the possible roles for geographical, regional or race links between the p53 gene polymorphism and the risk of breast cancer. It is worthy to indicate that recent studies showed a decrease in the average age to suffer from breast cancer in Iran [20]. Although life expectancy has been increased in parallel to decreasing of mortality, the overall prevalence has been mounted [21]. The present study therefore, aimed to determine such possible effect of having a specific polymorphism in codon 72 of p53 gene including arginine/arginine, proline/proline, or arginine/proline genotypes on the prevalence status of cancer and as risk factors in breast cancer.

The authors followed up all patients for 30 months to examine that if there was any relationship between those polymorphic genotypes and survival rates because, to the best of their knowledge, there is no report in the region of such research. The prognosis value of determination of such polymorphisms and their utilization in early diagnosis and treatment, particularly when younger women are more prone to this cancer, were also examined.

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Materials and Methods

Patients and controls

The study was a descriptive, analytical case control which was conducted on 160 samples including 80 patients with breast carcinoma and 80 matched healthy controls. The ethical committee of the university approved the study. After describing the study's nature, aims and possible benefits for improvement to prevent or cure the disease a written consent form was given from each involved sample.

For pathological diagnosis of the breast carcinoma, five sections with five-µm thickness were prepared from each patient. A peripheral blood sample at 1.5 ml was taken from each healthy matched control, homogenized, and stored in the tubes containing 0.5 molar ethylene diamine tetra acetic acid (EDTA) at -20ºC.

DNA extraction and PCR

DNA was separately extracted from each patient and control using standard kit. Codon 72 of the p53 gene was amplified using two specific primers by PCR technique. Specific primers for proline and arginine were prepared and lyophilized. They were diluted using deionized sterilized water to the required weight/volume based on manufacturer's instructions. The five mmolar dNTP solution (stock ten mmolar) was used. The sequences for proline and arginine primers were as follows respetively:

Forward: 5'GCCAGAGGCTGCTCCCCC3' 3'
Reverse: 5'CGTGCAAGTCACAGACTT
Forward: 5' TCCCCCTTGCCGTCCCAA3' 3'
Reverse: 5' CTGGTGCAGGGGCCACGC

During the optimized PCR process 59ºC was used for 50 seconds to amplify proline and arginine. The concentrations of materials used were as follows: DNA at five mM, primers and chloride magnesium at 0.5 mM, arginine at two mM and proline at five mM. The exon 4 of the p53 gene containing codon 72 was amplified in 35 cycles at the same condition for all collected samples.

Histopathological experiments

All diagnosed cancerous tissues after surgery were fixed with 10% formalin and renewed after four hours. Tissue passage was done by a processing tissue device after 24 hours. Sections with five-µm thickness were prepared for all samples using rotational microtome. For background staining hematoxylin, eosin, a specific monoclonal rabbit antihuman PTEN antibody and avidin-biotinolated immunoperoxidase were used. To localize the antigenic determinants a citrate buffer at 0.9% and hydrogen peroxidase at 3% were added to all samples and kept at 37º C for 30 minutes to inhibit endogenous peroxidase. After five times washing with phosphate buffer saline (PBS), streptoavidin conjugated with horse radish phosphate (HRP) was added to all sections to oxidize diaminobenzidine (DAB) which stained cells to brown.

Grades and stages

For determination of grades and stages, all samples were examined by two separate pathologists. Microscopy was carried out using a motic microscope equipped with advanced motic plus software with both 100 and 400 magnifications [22]. Histological grading was separately performed by microscopy based on the three following parameters: mitotic activity, nuclear pleomorphism, and the extent of tubule formation. Grades were then proposed in three groups: 1, 2, and 3 [23]. Tumor stages in the breast cancer were designated in numbers from 0 to 4 when 0 is for in situ carcinoma and Stages I, II, III, and IV are for the four consequent stages [24].

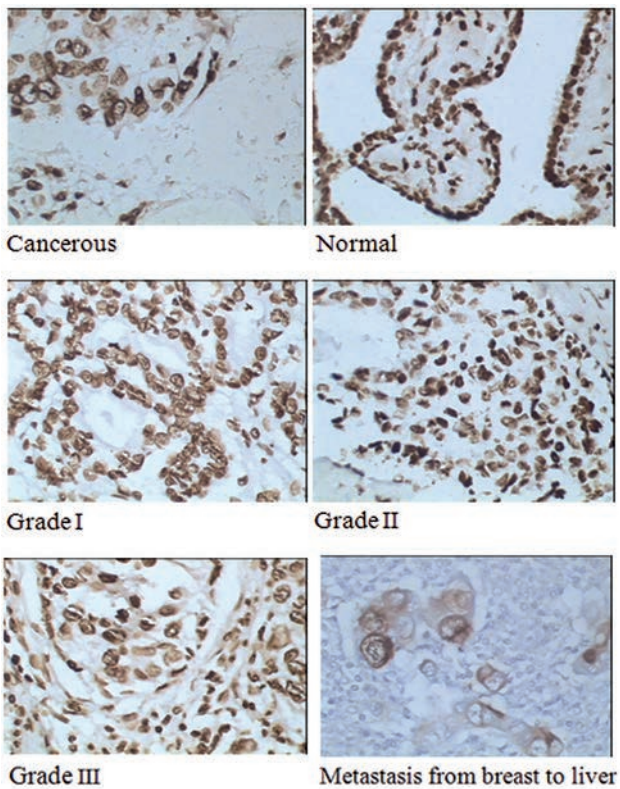

Figure 1. — Cross sections (four µm) from healthy controls and patients. Above: left cancerous, right normal. The following figures show different grades and metastatic section in the liver. All samples, normal and cancerous, were stained by H/E for the background and specific staining using streptoavidine conjugated with HRP which is able to oxidize (DAB). This staining method colored cells brown so that they can be distinguished with each other and other components. The magnification was 100 except for figures showed grades and metastatic pictures for which 400 magnification scales were utilized.

Electrophoresis

Electrophoresis was briefly set as follows: PCR product at five µl was mixed with one µl of loading dye and inserted into 1.5% agarose gel. For DNA staining, ethidium bromide was used and the results were photographed using gel documentation device. The genotype for each sample was then identified based on the base pairs (bp) of each bind compared with the standard marker.

Sequencing: Before sequencing each DNA sample was prepared by cutting the selected binds and was then amplified by PCR and the resultants were then sent to South Korea for sequencing.

Follow up: To evaluate relationship between the survival rate of patients with breast cancer and the genotypic mutation characteristics of codon 72, all patients were followed up for 30 months. For this purpose the authors contacted close relatives of all patients at proper intervals to ask and check the status of each one.

Statistics

Data was analyzed using SPSS software version 15. The Pearson chi-square and Fisher exact tests were used when required to explore associations between genotype and histological parameters. Using Kaplan Meier method, survival rate of patients with

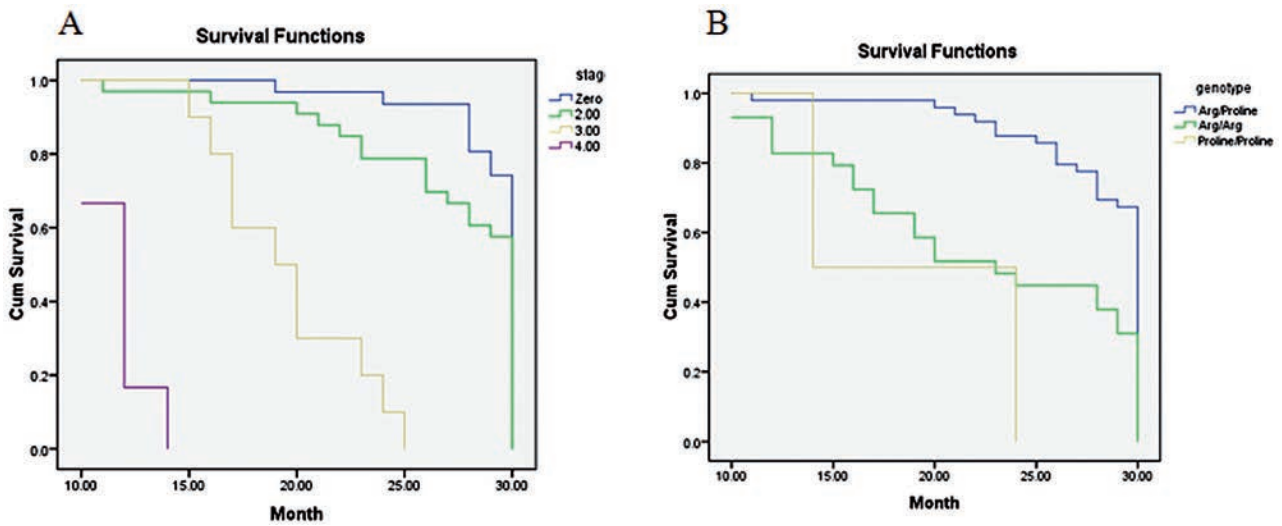

Figure 2. — The association between survival rates and the stages of cancer. For calculation Stages 0 and I were accumulated and designated as Stage I and the other as II, III, and IV. During follow up, the stages of the disease were measured and as expected patients with higher stages had lesser survival rate. A: Association between survival rates and the stages of the breast cancer. B: Association between survival rates and genotypes of the breast cancer. Genotype 1 was designated as arginine/proline, genotype 2 was designated as arginine/arginine, and genotype 3 was designated as proline/proline.

different genotypes and groups in higher and lower cancer stages was explored. Differences were tested by Mantel-Cox log-rank test. The results were considered to have significant difference when the p value was < 0.05 through all experiments.

Results

In the present study all 80 cancerous patients were diagnosed with a breast carcinoma. The minimum and maximum ages in patients were 20 and 86 years. In the healthy controls their ages were between 23 and 80 years. The average age for patients and controls were 47.22 ± 12.95 and 48.02 ± 12.48 years, respectively.

Stages

Of 80 patients, 31 (38.8%) cases were in the Stages of 0 (in situ carcinoma) and I who had cancerous cells limited to one or some lobules and ducts and there was no sign of metastasis in fatty tissues, lymph nodes or cells surrounding the location of the carcinoma. There were 33 (41.3%) samples in Stage II. They showed metastasis in the nearby tissues such as close lymph nodes. The number in Stage III was ten (12.5%) with metastatic cells in the regional lymph nodes. The other six patients had carcinoma in Stage IV and metastasis was observed in three patients in their lungs, one in her second breast and the remaining two ones in their liver (Figure 1).

Grades

There were 13 (16.3%) samples with grade 1, 45 (56.3%) with grade 2, and 22 (27.5%) with grade 3 (Figure 2). Regarding the grade and the menopausal age, the results showed significant differences between women who were at or less than 45 years old and older than 45 years. For example, a significant difference ($p < 0.007$) was seen between ten (12.5%) non-menopausal women and in three (3.8%) menopausal ones with grade 1. There were 22 (27.5%) non-menopausal and 23 (28.8%) menopausal cases with grade 2. However, for all women with grade 3, 17 (21.4%) menopausal ones had significantly higher rate ($p <$

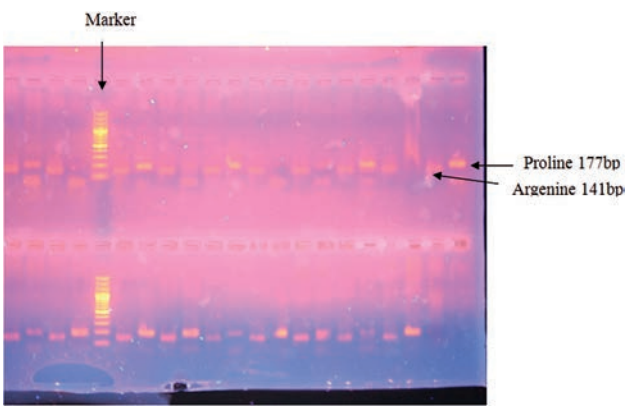

Figure 3. — The PCR products of codon 72 of p53 gene in exon 4 for proline/proline, arginine/arginine and proline/arginine in samples with breast cancer. The marker ladder was a standard ladder of 100 bp. The allele for proline/proline was 177 bp.The allele for arginine/arginine was 141.

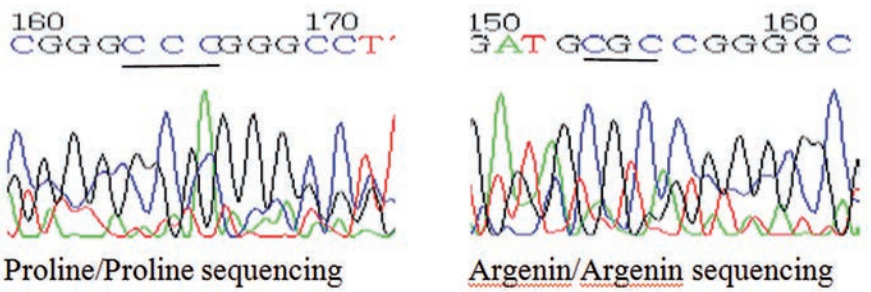

Figure 4. — The results for identifying sequencing of codon 72 polymorphism of p53 gene. Left above: The figure shows the genotype CGC which encodes proline. Right above B: The figure shows the genotype CCC which encodes arginine.

Table 1. — *The frequency of the genotype codon 72 of p53 gene expression in the different tumor stages in the cells of breast cancer and normal tissue. The data were statistically tested to find any significant differences.*

Variables	Arg/pro No. (%)	Arg/Arg No. (%)	Pro/Pro No. (%)	Statistical relationship
Cancer	49 (30.6%)	29 (18.1%)	2 (1.3%)	$p < 0.001$
Normal	51 (31.9%)	15 (9.4%)	14 (8.8%)	
Stage of cancer in situ carcinoma				$p < 0.001$
and I	22 (27.5%)	9 (11.5%)	0 (0%)	
II	26 (32.5%)	7 (8.8%)	0 (0%)	
III	1 (1.3%)	8 (10%)	1 (1.3%)	
IV	0 (0%)	5 (6.3%)	1 (1.3%)	
Tumor grade				$p < 0.01$
I	7 (8.8%)	6 (7.5%)	0 (0%)	
II	34 (42.5%)	11 (13.8%)	0 (0%)	
III	8 (10%)	12 (15%)	2 (2.5 %)	
Age < 45	55 (34%)	20 (12.5%)	12 (7.5%)	$p = 0.124$
Age > 45	45 (28%)	24 (15%)	4 (2.5%)	

0.007) compared with five (6.3%) non-menopausal women. There were no significant differences ($p < 0.44$) between the stage of the carcinoma and either non-menopausal or menopausal situation.

The association between the survival rates in the patients with arginine/proline genotype was higher than that in those who had arginine/arginine genotype. Patients with proline/proline genotype had the lowest survival rate compared with other checked genotypes (Figure 2).

Genotypic polymorphism

The authors analyzed the frequency and distribution of different arginine and proline genotypes. Their alleles had 141 and 177 base pairs respectively. The frequency of heterogeneous arginine/proline genotype was 49 (30.6%) and 51 (31.9%) in the patients and healthy controls, respectively, with no significant differences. The frequency of arginine/ arginine was 29 (18.1%) in the cancerous samples whereas it was 15 (9.4%) in the healthy controls, which showed a higher rate in the patients compared with the controls but, there was no significant difference. However, the frequency of homogeneous proline/proline genotype in the healthy controls with 14 (8.8%) cases was significantly higher than that in the patients with two (1.3%) cases ($p < 0.001$)(Table 1). Below we have brought the genotypes followed by their consequent stages in accordance with their frequency: proline/proline at Stage IV arginine/arginine at Stage III, arginine/proline at Stage II<, and Stage I, respectively. The results for bands of arginine and proline alleles are shown in Figure 3.

Sequencing results

The results of sequencing for homozygous arginine/arginine, proline/proline, and heterogenous arginine/arginine were consistent with the results given by PCR and electrophoresis in the patients and healthy controls (Figure 1). Moreover, the results of grading and staging were also consistent in terms of having significant or non-significant differences between genotypes in the patients and controls (Figure 4).

Follow up

Association between the survival rate and the genotypic mutation characteristics of codon 72 of p53 gene were examined for all patients. All patients were followed up for 30 consequent months by interval contacts with their closed relatives. It was expected that patients who were in higher stages had lesser survival rate compared to those who were in lower stages. During follow up, 38 patients out of 80 ones died. Of 38 dead 20 had hemozygote arginine/arginine, 16 had heterozygote arginine/proline, and two patients had proline/proline genotypes. The highest survival rate was observed in patients with arginine/proline genotype compared with others. The second higher survival rates were for cases with arginine/arginine whereas two patients with proline/proline genotype had the lowest survival rate among the examined cases though they were only two cases.

Discussion

Studies have shown that the risk of breast cancer mortality may be affected by genetic and epigenetic factors [25] including polymorphisms in the p53 gene. In this gene codon 72 on exon 4 have two distinct alleles which encode arginine (CGC) and proline (CCC), respectively, in the p53

protein structure [26]. The present results showed that in the healthy people, the homozygote proline/proline was significantly higher compared to the patients. In contrast, the homozygote arginine/arginine genotype in the patients with breast carcinoma was higher compared with the healthy controls. The authors also found that there was slightly higher heterozygote proline /arginine in the healthy people compared with the patients.

The survival rate in the patients with arginine/proline genotype was higher than that in those who had arginine/arginine. So, it seems that patients with arginine/arginine had more chance to suffer from cancer. Cases with proline/proline genotype had the significantly lowest survival rate compared with other checked genotypes. However, it may be assumed that, the reason could be the number of the cases as they were only two. More importantly they showed the highest stages, one at the stage III and another one at the stage IV. These findings suggest that we need to perform more investigations with a bigger sample size to cover other possibilities**.**

The significant association between the grades and the stages with the genotype was also observed. However, there was no such association in women regarding menopausal or non-menopausal status and the distinct genotype. Some studies in Brazil and Greece were in agreement with the present results as they reported a significant link between the presence of arginine/arginine and the risk of the breast cancer [15]. In contrast, Vijayraman *et al.* from Maduria have reported that there was no such link between the arginine/arginine, proline/proline, and arginine/proline genotypes of p53 gene and the breast cancer [27]. However, it is worthy to note that in that study, sample size was smaller than the present. They had only 100 samples (50 patients and 50 controls) whereas the present authors examined 160 people, including 80 with cancer and 80 that were healthy.

An association between proline/proline genotype for exon 4 of codon 72 has been shown by a group in Austria [28] which demonstrated similar findings with the present results. They also showed that the frequency of heterozygous proline/arginine was close to each other in the cancerous and healthy people. However, unlike the present study, they suggested that proline/proline had a role in breast cancer. In Japan, it has been also reported that people with proline/proline for codon 72 of p53 gene had lesser life expectancy than those with either proline/arginine or arginine/arginine genotypes [29]. Again in agreement with the present results, a study in Saudi Arabia claimed that there was a positive link between the initiation of the breast cancer and the arginine/arginine genotype. In addition, they proposed that there is a possible protective role for arginine/proline genotype [30].

According to above studies, it may be suggested that the stage status of the disease had more important role in the survival rate compared to the genotype status (Figure 4). These controversial results may reflect the possibility of different functionality for genotypic polymorphism of codon 72 which may be influenced by other factors, such as geographical and regional epigenetic specifications or even other unknown reasons. Further investigations, therefore, may be required to clarify and justify such controversies. In addition, these results are the reason why many studies have focused on the roles of codon 72 of p53 gene worldwide, particularly in the case of breast cancer. Similar to the present results in another study, Gochhait *et al.* showed that arginine/arginine genotype was more prevalent in the women with breast cancer compared with healthy ones [31].

Moving to another insight, it has been also shown that responses to the breast cancer treatment with anthracycline can be influenced by polymorphism of codon 72 of p53 gene in addition to pathological characteristics [25]. In this regard Wegman *et al.* in Sweden have reported that patients with breast cancer who had estrogen receptors and proline allele had shown better response to tamoxifen against the breast cancer than those who carried other alleles of codon 72 [32]. They also showed that patients who had arginine/arginine genotype had the same response to the treatment with either tamoxifen or non-tamoxifen chemotherapy. Therefore, these results suggested that lack of proline allele in the patients resulted in the better effective response to non-tamoxifen chemotherapy [32]. Schneider *et al.* who worked on neck and head cancers in Germany believed that the role of polymorphism of codon 72 of p53 gene is due to its role in apoptosis in malignant cells. Their study showed that tumors with arginine/arginine were resistant to apoptosis while tumors with proline/arginine were susceptible to the same apoptosis process [33]. Like the present results, their study confirmed the importance of some aspects of oncogenesis for arginine/arginine allele.

Conclusion

Controversial findings in breast cancer studies in different regions [34] suggested that there are probably regional patterns for the appearing and presence of a particular genotype of codon of 72, which plays role in oncogenic processes. The present authors may assume that, due to the effect of epigenetic factors in genotypic changes and consequently in different functions, more experimental wider works are required to identify the genotypic situation of codon 72 in any area where breast cancer is endemic and prevalent. The importance of breast cancer, the realty that there are no sufficient studies to clarify possible roles of polymorphism in codon 72 in different regions and populations, and many other controversial results, would encourage researchers to design comprehensive studies focusing on the factors affecting the survival rate, life expectancy, and related regional factors. These findings may lead to earlier diagnosis and subsequently the findings would be the best effective prevention and treatment protocols and hence would lead to less mortality due to breast cancer.

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Concomitant chemoradiotherapy versus pure radiotherapy in locally advanced cervical cancer: a retrospective analysis of complications and clinical outcome

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Summary

Purpose: To assess the complications and clinical outcomes of concurrent chemoradiotherapy (CCRT) or pure radiotherapy (RT) in local advanced cervical carcinoma (LACC) patients. *Materials and Methods:* A retrospective study was carried out in 113 consecutive LACC (FIGO Stage IB2-IIIB) patients, of whom 68 received CCRT; the others received pure RT. Five-year overall survival (OS) and the incidence, type, and severity of postoperative complications were analyzed. *Results:* The five-year survival rate for CCRT and pure RT were 67.7% and 46.8%, respectively ($p = 0.018$). The incidences of bone marrow suppression and gastrointestinal reaction for CCRT and pure RT were 100% *vs.* 88.89% ($p < 0.001$) and 70.6% *vs.* 33.33% ($p < 0.001$). Only 16.18% patients received CCRT developed chronic radiation enteritis, and 4.35% developed chronic radiation cystitis. While 11.11% patients received pure RT experienced chronic radiation enteritis ($p = 0.449$), 4.44% experienced chronic radiation cystitis ($p = 0.312$). *Conclusions:* This retrospective study demonstrated that CCRT followed by radical surgery achieved a better outcome compared with pure RT in LACC patients, but could apparently rise the incidence and severity of hematologic and gastrointestinal toxicity.

Key words: Local advanced cervical carcinoma; Concurrent chemoradiotherapy; pure radiotherapy; Complications; Clinical outcome.

Introduction

Cervical carcinoma is the second most common cancer affecting women's health worldwide, and more than 80% of all cervical cancers occur in women in developing countries [1]. Cervical cancer remains an important public health problem in mainland China. In 2005, there were approximately 58,000 new cervical cancer cases (National Office for Cancer Prevention and Control *et al.*, 2009) and about 20,000 deaths [2].

The treatment strategy of cervical carcinoma has been improved significantly in the past two decades. On the basis of numerous notable studies in the late 1990s, concurrent irradiation with cisplatinum-based chemotherapy (CCRT) has been recommended as standard treatment for local advanced cervical carcinoma (LACC) in most developed counties in the world [3, 4]. Cisplatin added to radiation could reduce the relative risk of death from cervical carcinoma by approximately 50% by decreasing local failure and distant metastasis, and improve overall survival (OS) by 9%–18% as well [5, 6]. However, the five-year OS of LACC patients still remains around 70% [7], and in elderly patients, patients with co-morbid medical conditions, poor performance status (PS), and those who refused chemotherapy cannot be administered, for which a different strategy is required to enhance the effects of radiotherapy given as a single modality of treatment [8]. Moreover, one of the major criticisms raised against CCRT is the potentially higher risk of complications, such as hematologic and gastrointestinal toxicities. Thus, it is still critical to explore a more effective therapeutic strategy for further OS improvement of LACC.

Until now, it is still not clear whether CCRT can provide a significant advantage for LACCs, eg, Stages III–IVA, in comparison with pure radiotherapy (RT) or in combination following radical surgery [9]. A meta-analysis study demonstrated that survival benefit of CCRT might be restricted to lower stage patients with International Federation of Obstetricians and Gynaecologists (FIGO) Stage IB–IIA, IIB having an increase in OS of 10% and 7%, respectively, by CCRT [10]. A retrospective study carried out in 174 Chinese patients with LACC reported that preoperative CCRT achieved outcomes superior to RT alone, but depending on the pathologic response, tumor size and lymph-node involvement as major prognostic factors [11]. However, previous studies did not define the incidence, type, and severity of postoperative complications and long term efficacy of CCRT in a large series of Chinese LACC patients. Therefore, the aim of this study was to determine

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Table 1. — *Comparison of baseline clinical characteristics between the two groups.*

Clinical variables	Group A (n=68)	Group B (n=45)	*p*
Median age (range)	47.5 (29.3-64.2)	48.6 (31.6-67.8)	0.674
Histotype			
Squamous	58	41	
Adenocarcinoma	8	4	0.602
Adenosquamous carcinoma	2	0	
FIGO Staging			
IB2	21	16	
IIB	30	22	0.705
IIIB	17	7	

Group A: concomitant chemoradiotherapy; Group B: pure radiotherapy. FIGO: The International Federation of Gynecology and Obstetrics.

whether CCRT offered a lower incidence of toxic reactions and better long-term efficacy in comparison with pure RT in a five-year follow-up retrospective cohort.

Materials and Methods

Patients

LACC patients who were treated in The Second Hospital of Tianjin Medical between January 2007 and January 2009 were recruited in this study. Inclusion criteria were: (1) Biopsy proven cases of advanced squamous cell carcinoma of uterine cervix, Stage IB2–IIIB (as per FIGO 2009 staging); (2) age between 25 and 75 years; (3) Karnofsky Performance Status (KPS) ≥ 70; (4) adequate bone marrow, liver, and renal function (Hb ≥ 10 g/dl; WBC ≥ 3,000/mm^3, platelets ≥ 120,000/mm^3; bilirubin < 2 mg/dl; blood urea nitrogen < 25 mg/dl, creatinine < 1.5 mg/dl); (5) no obvious mental abnormalities, psychological disorder and cognitive impairment; (6) with complete basic medical records and follow-up information. Exclusion criteria were: (1) age > 75 years or < 25 years; (2) KPS < 70; (3) pregnancy; (4) history of pelvic surgery, malignancy, exposure to cytotoxic chemotherapy or radiation; (5) combination of other tumor or sever chronic illness, such as chronic obstructive pulmonary disease, coronary heart disease, diabetes; (6) with distant metastasis. The study was approved by the Institutional Review Board of the present hospital. Written informed consent was obtained from all of the patients according to the committee's regulations.

One hundred thirteen LACC cases who met the eligibility criteria were divided into two groups according to the treatment arm: 68 patients in group A received CCRT, and 45 patients in group B received pure RT. Baseline patient characteristics were similar and well-balanced in both groups (Table 1).

Treatment

Both groups received combination of external beam radiation therapy (EBRT) and intracavitary brachytherapy (ICBT). EBRT included 6~15 MV of linear accelerator therapy apparatus, and ICBT included a WD- HDR18 close after installed with Iridium 192 radioactive sources. EBRT included DT 46~50 Gy, 2.0 Gy/times, five times a week, and the radiation field included the upper bound on the edge between the lumbar spine, lower obturator under two cm, and with the vaginal invaded scope changes, the lateral reach derma pelvic most outside diameter 1~2 cm wide, common iliac, external iliac, and iliac and sacral front and obturator lymph nodes were involved. All patients received ICBT immediately after completion of EBRT, DT 36~42 Gy, six Gy/week,once a week to point A (a point two cm lateral to the center of the uterine canal and two cm above the mucous membrane of the lateral fornix of the vagina in the plane of the uterus). The CCRT group received RT. Chemotherapy began from the first day of RT using PF scheme: cisplatin (DDP) 50~70 mg/m^2 1~2 days, 5 fluorouracil (5-Fu) 750 mg/m^2, 2~5 days, intravenous drip, three weeks/times, a total of three times.

Follow-up and toxicity evaluation

Patients were followed up by both the radiation oncologist and the gynecologist with detailed physical and gynecological examinations. Patients were followed up every six months from June 2009 to June 2014. The incidence of bone marrow suppression, gastrointestinal reaction, chronic radiation enteritis, and chronic radiation cystitis were assessed and recorded. Toxicity assessment was performed according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment for Cancer late-radiation morbidity-scoring scheme [12].

Statistical analysis

Statistical analysis was performed with SPSS 18.0, χ^2 test were used for categorical variables, and Mann-Whitney U test was used to for continuous values. OS was calculated from the date of diagnosis to the date of death or the date of the last follow-up. Survival curves were plotted using the Kaplan-Meier product-limit method, and differences between survival curves were tested using the log-rank test. All tests were two-tailed and a *p*-value < 0.05 was considered significant.

Table 2. — *Comparison of the incidence of bone marrow suppression between the two groups (n / %).*

Groups	Cases	Grade 0	Grade I	Grade II	Grade III	Grade IV	Total	Z	*p**
Groups A	68	0 (0.00)	12 (17.65)	37 (54.41)	15 (22.06)	4 (5.89)	68 (100)	-4.879	0.000
Groups B	45	54 (11.11)	21 (46.67)	17 (37.78)	2 (4.44)	0 (0.00)	40 (88.89)		

Group A: concomitant chemoradiotherapy; Group B: pure radiotherapy. *Mann-Whitney U test.

Table 3. — *Comparison of the incidence of gastrointestinal reaction between the two groups (n / %).*

Groups	Cases	Grade 0	Grade I	Grade II	Grade III	Grade IV	Total	Z	*p**
Groups A	68	20 (29.4)	20 (29.4)	12 (17.6)	10 (14.7)	6 (8.82)	48 (70.6)	-3.788	0.000
Groups B	45	30 (66.67)	7 (15.56)	5 (11.11)	2 (4.44)	1 (2.22)	15 (33.33)		

Group A: concomitant chemoradiotherapy; Group B: pure radiotherapy. *Mann-Whitney U test.

Table 4. — *Comparison of the incidence of chronic radiation enteritis between the two groups (n / %).*

Groups	Cases	Grade 0	Grade I	Grade II	Grade III	Grade IV	Total	Z	p^*
Groups A	68	57 (83.82)	4 (5.89)	5 (7.35)	2 (2.94)	0 (0.00)	11 (16.18)	-0.799	0.449
Groups B	45	40 (88.89)	3 (6.67)	1 (2.22)	1 (2.22)	0 (0.00)	5(11.11)		

Group A: concomitant chemoradiotherapy; Group B: pure radiotherapy. *Mann-Whitney U test.

Table 5. — *Comparison of the incidence of chronic radiation cystitis between the two groups (n / %).*

Groups	Cases	Grade 0	Grade I	Grade II	Grade III	Grade IV	Total	Z	p^*
Groups A	68	63 (92.65)	3 (4.41)	1 (1.47)	1 (1.47)	0 (0.00)	5 (4.35)	-0.625	0.612
Groups B	45	43 (95.56)	1 (2.22)	1 (2.22)	0 (0.00)	0 (0.00)	2 (4.44)		

Group A: concomitant chemoradiotherapy; Group B: pure radiotherapy. *Mann-Whitney U test.

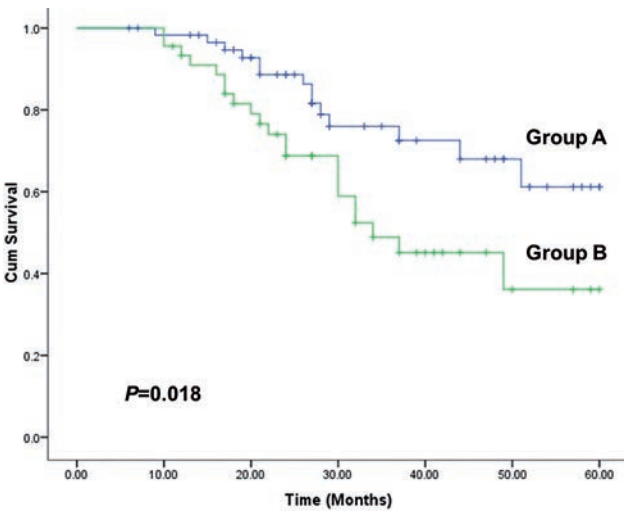

Figure 1. — Overall survival by means of the Kaplan–Meier survival analysis using the Log rank test. The overall survival time was 48.8 months for group A and 39.2 months for group B. The five-year survival rate of the group A was significantly higher than the group B (76.7% *vs.* 53.3%; $\chi^2 = 5.629$, $p = 0.018$).

Results

Hematologic, gastrointestinal, nephrotoxic. and urinary complications were the most common types of toxicity. During the observation period, as shown in Table 2, 68 (100%) patients in group A experienced no grade bone marrow suppression complications, and 19 (27.94%) of them had ≥ grade 3 complications. In Group B, the bone marrow suppression complications occurred in 40 (88.89%) patients, significantly lower than that of group A (Z = - 4.579, $p < 0.000$), and two (4.44%) of them had ≥ grade 3 complications, significantly lower than that of group A ($\chi^2 = 8.389$, $p = 0.004$).

As shown in Table 3, the incidence rate of gastrointestinal reaction in group A was 70.6% (48/68), significantly higher than that of group B [33.33% (15/45), Z = - 3.788, $p < 0.000$]. The III-IV grade gastrointestinal reaction in group A was 23.53% (16/68), significantly higher than that of group B [6.67% (3/45), $\chi^2 = 4.336$, $p = 0.037$].

Of the 68 patients in group A, only 11 (16.18%) patients developed early-grade chronic radiation enteritis, and five (4.35%) patients developed early-grade chronic radiation cystitis. While in group B, five (11.11%) patients developed early-grade chronic radiation enteritis, and two (4.44%) patients developed early-grade chronic radiation cystitis. No significantly differences were observed between two groups (Z=-0.799, $p = 0.449$; Z = - 0.625, $p = 0.312$, respectively) (Tables 4 and 5).

No patients were lost during the follow-up; the median follow-up was 52 months in both groups. The overall survival time was 48.8 months for group A and 39.2 months for group B. The five-year survival rate of the group A was significantly higher than the group B (67.7% *vs.* 46.8%; $\chi^2 = 5.629$, $p = 0.018$) (Figure 1).

Discussion

This retrospective study evaluated the prevalence of complications and long term-efficacy of CCRT in a relatively large sample of Chinese LACC patients. The results demonstrated that CCRT achieved an outcome superior to pure RT in 113 Chinese patients with LACC, which was consistent with previous studies [11]. However, the present study found that preoperative CCRT was associated with significantly higher incidence of bone marrow suppression and gastrointestinal reaction, compared with RT alone, suggesting that CCRT could increase the hematologic and gastrointestinal toxicity.

CCRT is now the standard treatment in LACC and cisplatin appears to be the ideal chemotherapeutic agent. Green *et al.* [13] analyzed data from 19 randomized trials comprising 4,580 patients and concluded that concomitant chemotherapy results in improved overall survival and progression-free survival. However, the absolute survival benefit was 12% maximum in early-stage (I and II) disease, and the three-year overall survival (74%) or five-year overall survival or progression-free survival (50%–63%) of the standard CCRT alone were still not satisfactory [14]. In the present study, the five-year OS rates for patients undergoing CCRT and pure RT were 67.7% and 46.8%, respectively, suggesting that preoperative CCRT achieved better outcome in comparison to RT alone for LACC with acceptable low nephrotoxic and uri-

nary toxicity and complications. This study together with previous studies suggest that a combination of preoperative CCRT and radical surgery may provide a feasible and effective treatment for patients with LACC.

In the present study, patients receiving CCRT all experienced bone marrow suppression complications, and 27.94% had grades 3 and 4 in comparison to RT alone (4.44%, $p = 0.004$). Morris *et al.* found that 44% patients receiving pelvic radiation with concurrent chemotherapy experienced grades 3 and 4 bone marrow suppression complications, while this only occurred in 3% patients treated with RT alone [15]. In addition, 70.6% patients receiving CCRT experienced gastrointestinal reaction, and 23.53% had grades 3 and 4 in comparison to RT alone (6.67%, $p = 0.037$). Green *et al.* also reported that the incidence of gastrointestinal reaction was significantly higher in CCRT patients compared with RT patients [16]. Several large cohort and phase-III studies on exclusive CCRT have also described severe late toxicity ranging from 10% to 18.3 % with a predominant pattern of intestinal toxicity (13% grades 3–4 complications) and vaginal toxicity (20% grades 3–4 complications) [17, 18]. Interestingly, CCRT treatment showed similar toxicities compared with the RT treatment in chronic radiation enteritis and cystitis. The chronic radiation enteritis rates following radiation therapy range from 10-20%, and 1-10% for chronic radiation enteritis, depending on the bias of the reports. In the present study, 16.18% patients receiving CCRT developed chronic radiation enteritis, and 4.35% patients developed chronic radiation cystitis, and the results were in agreement with the above reported. Altogether, these studies suggest that CCRT could apparently rise the incidence and severity of hematologic and gastrointestinal toxicity, thus emphasizing the need of a close clinical and radiological monitoring of patients in postoperative period.

In conclusion, this retrospective study demonstrated that CCRT followed by radical surgery achieved a better outcome compared with pure RT in LACC patients, but could apparently increase the incidence and severity of hematologic and gastrointestinal toxicities. However, this finding is not conclusive due to the small sample size and lack of correlation analysis of clinical variables with complications, which are major drawbacks of this study. Moreover, the present authors indicate that this observation is still a retrospective study, which might be a limitation along with a lack of sufficiently balanced numbers of patients. However, these findings warrant further multicenter investigation in a randomized clinical trial.

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Incidence of inactive allele CYP2D6*4 among Greek women suffering from hormone-sensitive breast cancer

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Summary

Background: The incidence of CYP2D6*4 among Caucasians is estimated up to 27%, while it is present in up to 90% of all poor metabolizers within the Caucasian population. The hypothesis under question is whether the presence of one or two non-functioning (null) alleles predicts an inferior outcome in postmenopausal women with breast cancer receiving adjuvant treatment with tamoxifen. The aim of the present study is to estimate the incidence of CYP2D6*4, in the Greek population and more precisely among females suffering from breast cancer. *Materials and Methods:* Eighty unrelated mainland Greek female volunteers suffering from hormone-sensitive breast cancer were recruited during their primary handling or follow-up examination in order to provide samples for purification and polymerase chain reaction/ restriction fragment length polymorphism (PCR-RFLP) of genomic DNA derived from buccal swabs. *Results:* The incidence of individuals with at least one present allele*4 within the Hellenic population was estimated to be as high as 30% (n = 24/80), with a 95% confidence interval of 20% to 40%. From the statistical point of view, it can be securely stated that incidence of *4 among Greek women is over 20%. The incidence of homozygous carriers of *4 in the present sample occurred in 8.75%, while the incidence of allele*4 haplotype occurred in 19.4% (n=160). *Conclusion:* Although the outcoming results for Greek women are actually in line with existing data for other European nations, it should be noted, that a routine CYP2D6 testing of women suffering from breast cancer is formally not recommended, as the clinical significance of CYP2D6 phenotype in treatment and outcome of breast cancer remains unclear.

Key words: CYP2D6*4; Breast cancer; Tamoxifen; Caucasian.

Introduction

Cytochrome P-450 2D6 (CYP2D6) is of great clinical relevance, because it represents one of the most important enzymes involved in drug metabolism in general. The gene encoding this enzyme is highly polymorphic, as 93 alleles with varying function have been reported. According to the genotype, pattern individuals can be divided into four phenotype groups: ultra-rapid (UMs), extensive (EMs), intermediate (IMs), and poor metabolizers (PMs). UMs carry three active alleles (duplication or amplification effect); EMs are characterized by the presence of two functional alleles (*1, *2, *9); IMs carry only one active allele, while PMs express two inactive alleles (*3,*4,*5) [1].

In general, Caucasians have a quite higher incidence of the PM phenotype when compared to other races. The studies referring to African populations on the other hand show a wide range of results, with the South-Africans having an incidence of 19%. The lowest frequency is reported within the Asian population. Allele *3 and mostly *4, both of which are non-functional, are mainly responsible for the PM phenotype among Caucasians in general. On the contrary, these alleles are rarely found in the Asian population, explaining the worldwide lowest frequency of PM status in that group [2].

The role of CYP2D6 in the adjuvant treatment of breast cancer is crucial, as this enzyme is mainly involved in the biotransformation of tamoxifen to the potent antiestrogen endoxifen. The aim of the present study is to estimate the incidence of CYP2D6*4, in the Greek population and more precisely within females suffering from breast cancer. Despite the numerous existing studies focusing on the incidence of CYP2D6*4 between Caucasians in general, and among specific European ethnic groups as well, relevant data referring to the Greek race are missing. This is the first country-wide study attempting such an epidemiological screening approach.

Materials and Methods

A total of 80 unrelated mainland Greek female volunteers participated in the study after giving written informed consent. They were all patients suffering from hormone-sensitive breast cancer, that were recruited during their primary handling or follow-up examination at the Second Department of Propaedeutic Surgery of the Medical School in Athens. The study protocol was approved by the Ethics Committee of the Kapodistrian University of Athens Medical School. The patients were informed that the present study was only scheduled for statistical purposes and that the outcoming results would not influence their treatment regimen. The description of the patient's characteristics is presented in Tables 1 and 2.

Purification and polymerase chain reaction/ restriction fragment length polymorphism (PCR-RFLP) of genomic DNA was derived

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Table 1. — *Cohort descriptives I.*

	Total		CYP2D6=*4 No		Yes	
	N	%	N	%	N	%
Grade	14	17.5	11	19.6	3	12.5
I						
II	51	63.8	32	57.1	19	79.2
III	15	18.8	13	23.2	2	8.3
Histological type						
Papilar	1	1.3	1	1.8	.	.
Lobular	8	10	6	10.7	2	8.4
Mixed	2	2.5	2	3.6	.	.
Ductal	68	85.0	47	83.9	21	87.5
Hybrid	1	1.3	.	.	1	4.2

Table 2. — *Cohort descriptives II.*

		Total	CYP2D6=*4 No	Yes
ER- status	N	80	56	24
	Mean	0.7	0.7	0.8
	Median	0.8	0.8	0.8
	Min	0.2	0.2	0.2
	Max	1.0	1.0	1.0
PR-status	N	80	56	24
	Mean	0.6	0.6	0.7
	Median	0.7	0.6	0.7
	Min	0.0	0.0	0.0
	Max	1.0	1.0	1.0
Age (years)	N	80	56	24
	Mean	53.6	54.1	52.4
	Median	53.0	53.5	50.5
	Min	30.0	30.0	40.0
	Max	88.0	88.0	75.0

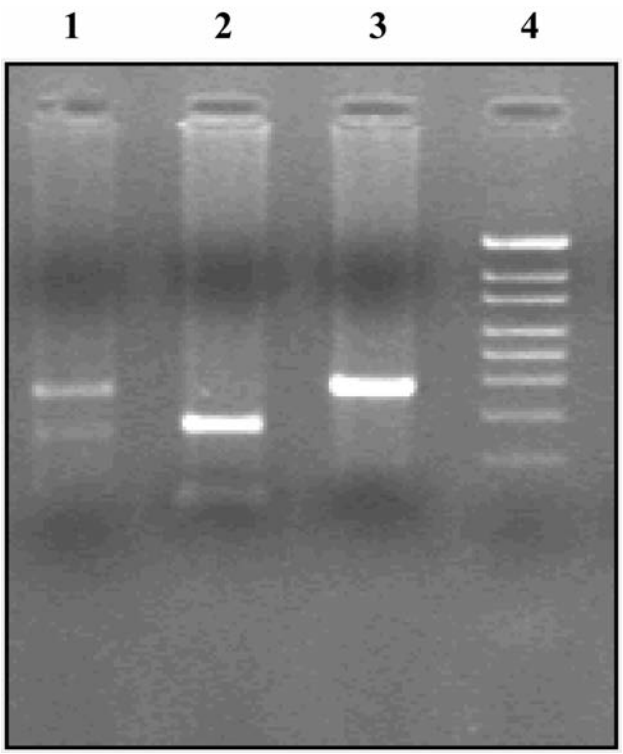

Figure 1. — Gel picture, CYP2D6*4 polymorphism.
Lane 1 – IM genotype (intermediate metaboliser -334, 230, and 104 base pairs).
Lane 2 – EM genotype (extensive metaboliser -230 and 104 base pairs).
Lane 3 – PM genotype (poor metaboliser -334 base pairs).
Lane 4 – 100 base pair ladder as marker.

from buccal swabs that was performed. The samples were collected with cotton swabs. The swab was scraped firmly against the inside of each cheek several times and was set to air dry. All individuals were informed to avoid consuming food or drink within 30 minutes prior to the collection of the sample. Each dry swab material was placed in a two-mL micro- centrifuge tube, where 300 µL PBS and 25 µL proteinase K solution was added. It followed a mix by vortexing 2x5 seconds and incubation for ten minutes at 56°C. The swab was at that point removed and 300 µL buffer B3 were added. The solution was vigorously vortexed and the sample was incubated at 70°C for another ten minutes. In order to adjust the DNA binding conditions 300 µL of 96%-100% ethanol were added to each sample and the new solution was once again mixed by vortexing. At that point 600 µL of the samples were transferred from the two-mL micro-centrifuge tubes into NucleoSpin Tissues Columns and was centrifuged at 12,000 x g for one minute. The prior added ethanol binds the DNA on to the column membrane. The flow-through was discarded and the columns were placed back into the collection tube. The silica membrane was initially washed after adding 500 µL of buffer BW and centrifuging for one minute at 4,500 x g. The second wash was performed with an addition of 600 µL buffer B5 to the column and centrifugation at 14,000 x g for two minutes. The flow-through was once more discarded. In order to remove the residual ethanol, the NucleoSpin Tissue Column were then placed into a new collection tube and were incubated with an open lid for one to two minutes at 70°C. In the next step and in order to elute highly pure DNA, the columns were placed into a 1.5-mL micro-centrifuge tube where 80 mL of pre-warmed elution buffer BE (70°C) were added. The solution was incubated for one minute and then centrifuged at 12,000 x g for one more minute. The quantitative measurement of the amount of isolated DNA could then be performed with photometry.

The investigation of the presence of CYP2D6*4 was performed using PCR-RFLP. For successful PCR, about five µl (200 ng) of DNA extract and 45 µl of PCR mix - including the two specific primers- were incubated under specific conditions. The used forward and reverse primers for CYP2D6*4 genotyping had the following nucleotide sequences: GCTTCGCCAACCACTCCG (CYP2D6-f) and AAATCCTGCTCTTCCGAGGC (CYP2D6-r). The 45 µl PCR mix contained five µl PCR-buffer w/o Mg, one µl dNTPs, 1.5 µl MgCl2, one µl of each of the primers 2D6-f and 2D6-r, 0.5 µl *Taq*-polymerase and 40 µl of H_2O. Thermocycling conditions were as follows: initial denaturation at 95°C for five minutes, 40 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 60 seconds. The terminal elongation was performed at 72°C for five minutes. If the PCR was successful (PCR product of 334 base pairs, checked by 2% agarose gel electrophoresis), 15 µl of the product was diluted with five volumes of distilled water and stored at 4°C. The PCR-product was then digested using the restriction endonuclease BstNI. The final digestion mix contained

Table 3. — *CYP2D6 genotyping results.*

CYP2D6	n	%
*4/*4	7	8,75
wt/*4	17	21.25
wt/wt	56	70.0

Table 4. — *Binomial proportion of CYP2D6*4.*

Proportion	0.3000
ASE	0.0512
95% lower conf. limit	0.1996
95% upper conf. limit	0.4004

15 μl of PCR product, five μl of NEB buffer, one μl of BstNI, and 29 μl H_2O (total mix volume 50 μl) and was incubated at 60°C for one hour. The digestion products were further analyzed on a 10% acrylamide gel electrophoresis, together with a 100-bp DNA weight marker. The excepted electrophoresis patterns and their interpretation are presented in Figure 1.

Statistical analysis

Sample size estimation was based on the assumption that the true incidence of allele *4 would approximately be 20%. The majority of the existing literature evidence that the incidence of allele *4 among healthy Caucasian women ranges from 18% to 21% [3, 4]. Thus with 80 patients (160 examined alleles), the expected level of confidence would be ± 7%. Asymptotic 95% confidence intervals where used in order to assess the level of accuracy for the point estimates, while hypothesis testing was used to test several alternatives.

Results

In the present study, the incidence of individuals with at least one present allele*4 within the Hellenic population was estimated to be as high as 30% (n = 24/80), with a 95% confidence interval of 20% to 40%. With this mean, it can be securely stated that incidence of *4 among Greek women is over 20%. Furthermore, the incidence of homozygous carriers of *4 in the present sample occurred on 8.75% (Tables 3 and 4), while the incidence of allele*4 haplotype occurred in 19.4% (n=160).

Discussion

The main question remaining to be answered is whether the routine use of CYP2D6 genotyping should be introduced in the adjuvant setting of tamoxifen or not. Focused on these issues while designing the present study, the sample consisted exclusively of Greek female patients with hormone-sensitive breast cancer.

A number of studies have estimated the incidence of CYP2D6 phenotype and the distribution of CYP2D6 alleles within Caucasians. Few of them are restricted to simple phenotype prediction as summarized in Table 5 [5], while others have specifically focused on the incidence of allele*4 in various European ethnic groups.

Table 5. — *Incidence of poor metabolizers (PM) within Caucasians [5].*

Population	PMs (%)
British	8.9
Swiss	10
German	7.7
Polish	8.3
Croatian	3.0

Table 6. — *Ethnic studies.*

Population	n	*4 incidence (%)	Population	n	*4 incidence (%)
Croatian [27]	200	14.0	Greek [21]	283	17.8
Croatian [26]	144	11.4	Italian [23]	350	15.3
Czech [12]	223	22.9	Norwegian [15]	118	20.0
Danish [19]	240	18.1	Polish [10]	145	23.1
Danish [20]	325	20.6	Polish [11]	300	23.0
Dutch [16]	756	18.4	Russian [18]	290	18.2
Estonian [13]	151	21.5	Russian [17]	204	14.4
Finnish [32]	302	12.8	Sardinian [22]	250	16.8
Finnish [31]	122	11.1	Spanish [30]	290	16.6
French [25]	514	18.6	Spanish [28]	258	12.2
French [24]	171	14.9	Spanish [29]	105	13.8
German [4]	589	20.7	Swedish [8]	281	24.4
German [14]	195	19.5	Swedish [9]	248	23.0

The activity of the CYP2D6 enzyme can be easily measured in vivo after the oral administration of a probe drug that is mainly CYP2D6 metabolized, such as dextromethorphan, debrisoquine or sparteine. The consequent estimation of the ratio of metabolite to parent drug concentration indicates the CYP2D6 metabolic status [6]. Regarding the detection of CYP2D6*4, it should be mentioned that, the standard nomenclature of the *4 allele is based on the presence of the 1846G>A defining variant. Furthermore, other haplotype variants could also be present [7].

The prevalence of the CYP2D6*4 allele, as estimated in the present study, complies with the Hardy-Weinberg equilibrium and is in line with the majority of published results for other European ethnicities of Caucasian origin. More precisely, the following frequencies have been reported among different Caucasian ethnicities: 24.4% - 23% in Swedes [8, 9], 23.1% - 23,0% in the Polish [10, 11], 22.9% in Czechs [12], 21.5% in Estonians [13], 19.5% and 20.7% in Germans [4, 14], 20.0% in Norwegians [15], 18.4% in Dutch [16], 14.4% - 18.2% in Russians [17, 18], 18.1% and 20.6% in Danish [19, 20], 17.8% in Greeks [21], 16.8% in Sardinians [22], 15.3% in Italians [23], 14.,9% - 18.6% in French [24, 25], 1.4% - 14% in Croatians [26, 27], 12.2%, 13.8% and 16.6% in Spanish [28-30] and 11.1% - 12.8% in Finish [31, 32]. The examined population and the concomitant incidence of CYP2D6*4 of the aforementioned reported studies are presented in Table 6.

Table 7. — *Multi-ethnic studies.*

Population	n	*4 incidence (%)
European [3]	672	18.9
European [33]	157	17.2
French	25	16.0
French Basque	24	20.8
Sardinian	28	21.4
North Italian	14	14.3
Tuscan	8	18.8
Orcadian	16	12.5
Adygei	17	8.8
Russian	25	20.0
Mediterranean [34]	247	16.0
Sardinian	48	12.5
Central Italians	31	12.9
Alps	28	19.64
Basques	38	21.05
Southern Spaniards	51	17.65

A special report should be also made on three large studies that analyzed the allele*4 incidence in multi-ethnic European cohorts (Table 7). Marez *et al*. analyzed 672 individuals of European origin and estimated the incidence of allele*4 to be as high as 18.9%. Further details regarding the sample composition are not available [3]. Similar results with an allele*4 incidence of 17.2% are also reported in the study of Sistonen *et al*. performed in a sample of 157 Europeans individuals [33]. The authors have additionally reported the respective frequencies in every ethnic group being part of their cohort. In one further large study within six populations of the Mediterranean region, the prevalence of allele*4 occurred in 16%. A further subanalysis of the allele*4 frequencies in each ethnic group has also been reported [34].

Four studies provide data about the incidence of CYP2D6*4 allele within Caucasians suffering from breast cancer. Bonanni *et al*. determined the CYP2D6 genotype in hysterectomized women participating in the Italian chemoprevention trial of tamoxifen. The frequency of the CYP2D6 *4/*4 genotype was statistically significant higher (9%) in women who developed breast cancer (n=46) than in the control group (n=136). The authors assumed that the expression of the inactive allele*4 may consist of a predisposing factor for breast cancer. A strong bias of their study is due to a lack of group-matching of the follow-up period and the risk factors associated with breast cancer as well [35].

Two further studies performed between Spanish individuals are giving conflicting results. Fernandez-Santanter *et al*. genotyped 96 breast cancer Spanish patients and compared them to 100 healthy control subjects. The incidence of allele*4 was 13.5% *vs.* 22% in patients and controls, respectively. Their results supported a statistically significant association between wild type CYP2D6 *vs.* homozygous *4 genotype and breast cancer risk [36]. This data is conflicting with the results of another study among Spanish individuals published by Ladona *et al*. Their cohort consisted of 151 breast cancer patients and 187 healthy controls. The authors supported an inverse relationship between CYP2D6 activity and breast cancer risk. The prevalence of heterozygous CYP2D6 (wt/*4) genotype was higher between individuals with breast cancer (26.7% *vs.* 17.2%, $p = 0.037$) [37].

Finally, Topic *et al.* compared the incidence of inactive allele*4 between breast cancer patients and healthy volunteers from Croatia [26]. The prevalence of CYP2D6*4 occurred in 18.4% among breast cancer subjects (28/152 tested alleles) *vs.* 11.4% among control individuals (33/288 tested alleles). The reported difference was furthermore not statistically significant, hence no association between CYP2D6 genotype and breast cancer risk could be safely supported .

In reference to the Greek population, the only previous existing study investigating the prevalence of various CYP2D6 genotypes within healthy Greeks has been published by Arvanitidis *et al.* [21]. In a total of 283 healthy subjects, 92 were detected to be carriers of the inactive allele*4. Eight of them were estimated to be homozygous (3.2%), while the frequency of allele*4 itself occurred in 17.84% (101/566 tested alleles). The present results, although exclusively based on breast cancer patients, are actually in line with those of Arvanitidis *et al*, so that no etiological relationship between CYP2D6 genotype and breast cancer risk among Greek women could be assumed. The extraction of such a conclusion is actually not safe and strongly biased, as the two cohorts were completely and independently analyzed and consisted of non-matched individuals.

The present authors refer to a prior review research of their institution, that evaluates the clinical implication of the non-functional allele *4 in breast cancer, always in regards to tamoxifen therapy [38]. The results were conflicting and quite inconclusive. Three former reports showed a favorable outcome in CYP2D6*4 carriers with ER+ breast cancer. These findings are actually opposed to the basic assumption and could not be supported by any other later study [39-41].

The great volume of published studies shows a clear negative relationship between intermediate/poor CYP2D6 metabolizing status and the outcome of ER+ breast cancer. This main hypothesis has also been supported from studies that have exclusively focused on allele*4 alone [35, 42-45]. Interestingly, the presence of inactive CYP2D6 alleles and in particular allele*4 has also been associated with lower circulating serum levels of tamoxifen metabolites. In that mean, the benefit of tamoxifen treatment is strongly limited in this patient group [46, 47]. On the other hand, the acceptance of such a negative impact of allele*4 in the course of breast cancer is not

unique. In a large population study published by Abraham *et al*., no association between CYP2D6 phenotypes (allele*4 included) and survival in breast cancer patients under tamoxifen treatment could be proven. Based on their results, the authors argued against CYP2D6 testing in the clinical setting [48].

Although the current recommendations for breast cancer treatment do not support a CYP2D6 screening prior to tamoxifen treatment, the interpretation of every existing result should be made with respect to the special parameters of each population.

The estimated incidence of CYP2D6*4 among Greek females suffering from breast cancer is quite high in comparison to previous presented results for other ethnic groups of Caucasian origin. The present cohort included only women with hormone positive cancers, in an effort to maximize the accuracy of the present results for this patient group, in which actually the administration of tamoxifen is absolutely indicated. The outcoming results are in accordance to the Hardy-Weinberg equilibrium and can be considered as highly reliable given Greece's consistent and homogeneous population.

In the present study, the testing for CYP2D6 allele*4 was performed using germline DNA extracted from hosts' buccal-derived sample. In a large number of published studies that doubt about the clinical significance of allele*4 in the treatment with tamoxifen, the genotyping procedure was performed at paraffin-fixed cancer tissue. It should be mentioned that tumor DNA may show significant differences from germline DNA due to "loss of heterozygosity" during cancer progression, a fact that depicts a strong bias of all these studies.

The cost effectiveness parameter is of high importance, given the long required time of tamoxifen administration in relation to the strong financial limitations of the Greek health system in an area of financial crisis. In patients where impaired function of CYP2D6 is expected, due to the presence of one or two *4 alleles, dose adjustment or other therapy regime should be considered. In patients which have been found to be intermediate or poor metabolizers, caution should be also given in any potential CYP2D6 inhibitors that may be occasionally co-prescribed due to other medical reasons [49]. If the administration of tamoxifen should be continued, dose reduction or alternative medication for the handling of co-morbidities might be indicated. Finally, patients that are not likely to benefit from a treatment with tamoxifen should also not be exposed to its possible various side and adverse effects [50, 51].

Conclusion

CYP2D6*4 is the most frequent allele associated with loss of enzymatic activity among Caucasians. The present study, performed on an ethnic basis, focused only on women with hormone-sensitive breast cancer, a patient group in which the administration of tamoxifen is absolutely indicated. The outcoming results for Greek women are actually in line to existing data of other European nations. Nevertheless it should be noted, that a routine CYP2D6 testing of women suffering from breast cancer is formally not recommended, as the clinical significance of CYP2D6 phenotype in treatment and outcome of breast cancer remains unclear.

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Outcomes of concurrent radiotherapy and weekly paclitaxel/carboplatin therapy in cervical cancer: a retrospective study

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Summary

Purpose of investigation: To determine if concurrent chemoradiotherapy (CCRT) with paclitaxel and carboplatin is effective, convenient, and tolerable for cervical cancer treatment. *Materials and Methods:* The authors retrospectively reviewed the medical records of 49 patients. Primary outcomes included progression-free survival (PFS) and overall survival (OS). The Cox proportional hazards model was adjusted for all prognostic factors in the multivariable analysis. *Results:* Over the median follow-up time of 32 months in a sample consisting of 87.8% (43/49) squamous cell carcinoma and 12.2% (6/49) adenocarcinoma, two-year PFS and OS rates were 67.2% and 80.9%, respectively. In univariate analyses, stage, histology, performance status, tumor size, and age were significant variables for OS; only histology was significant in the multivariable analysis. Acute toxicity grade 3 or 4 neutropenia (85.7%), diarrhea (32.7%), and late toxicity grade 3 or 4 (12.2%) were detected. *Conclusions:* For cervical cancer treatment, CCRT with paclitaxel/carboplatin is satisfactory.

Key words: Uterine cervical neoplasms; Chemoradiotherapy; Paclitaxel; Carboplatin; Survival.

Introduction

Cervical cancer is the one of most common causes of cancer-related death in women. Primary treatment currently includes radical surgery and radiotherapy. However, recent studies have shown that the curative effect of concurrent chemoradiotherapy (CCRT) is equivalent to radical surgery for early stage cervical cancer and more effective than radiation only [1-5].

Although the primary drug choice is cisplatin, paclitaxel or carboplatin alone has been shown to be efficacious for CCRT [6-8]. Carboplatin induces the same platinum-DNA adduct formation as cisplatin, is easy to use, and does not require hydration [9]; furthermore, it results in lower nephrotoxicity and emetogenicity than cisplatin. The combination of paclitaxel and cisplatin chemoradiation reportedly results in only mild toxicity and a good response rate in patients with locally advanced cervical cancer [10, 11]. The combination of paclitaxel and carboplatin is also effective as chemotherapy [12, 13], with a good survival rate [14]; as a result, this combination is used as chemotherapy for advanced or recurrent cervical cancer in Japan. In CCRT, these drugs act together as a radiosensitizer as well as effective chemotherapy.

The use of CCRT in other cancers has resulted in shorter treatment durations and improved efficacy in terms of progression-free survival (PFS), overall survival (OS), toxicity, and complications [15-19]. The present institute utilizes CCRT with paclitaxel and carboplatin because hydration and hospitalization are not required, and there are a limited number of hospital beds in this hospital [20]. However, the ideal approach for multimodal therapy that includes chemotherapy and external beam therapy for the treatment of cervical cancer has not yet been established. It is unknown if there is increased efficacy against cancer with the use of two antineoplastic drugs or with the administration of CCRT. Therefore, the present retrospective study aimed at evaluating CCRT with paclitaxel and carboplatin in a large sample of Japanese patients with cervical cancer.

Materials and Methods

With the approval of the Jichi Medical University Institutional Review Board, the authors retrospectively reviewed the medical records of patients who received CCRT with paclitaxel and carboplatin between September 2006 and June 2012 in the Department of Gynecology at the Saitama Medical Center Jichi Medical University. The need for informed consent was waived because data were only obtained via retrospective review of records.

Indications for CCRT included patients with International Federation of Gynecology and Obstetrics (FIGO) Stage IB2-IVB cervical cancer with histopathology of squamous cell carcinoma (SCC), adenocarcinoma, or adenosquamous carcinoma. Exclusion criteria included previous, partial treatment at another institution or history of another malignant disease.

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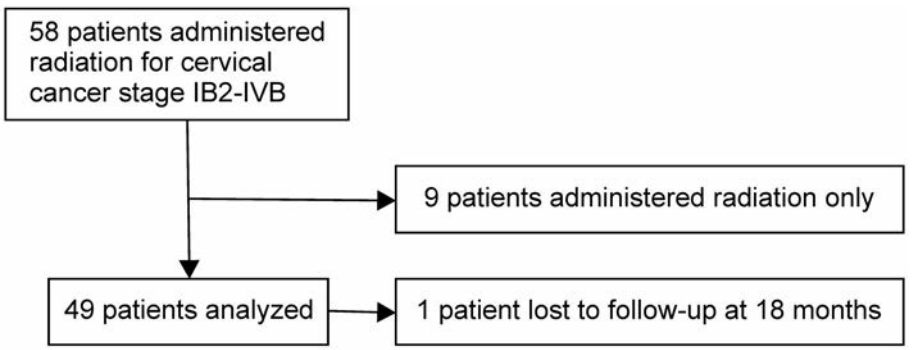

Figure 1. — Flowchart of enrollment of patients with Stage IB2-IVB cervical cancer.

The following data were collected: age, histopathology, stage, Eastern Cooperative Oncology Group performance status (PS), tumor size, number of chemotherapy cycles, toxicities, and tumor response, which was evaluated according to the Response Evaluation Criteria In Solid Tumors (RECIST) guideline (version 1.1). PFS and OS were determined as the primary outcomes. PFS was defined as the interval from the first date of diagnosis to the time of recurrence, disease progression, or death. PFS data were right-censored at the time of the last evaluation for patients lost to follow-up. OS was defined as the time from diagnosis to the date of death and right-censored at the date of the last follow-up visit for patients who were alive at the end of the study.

Clinical staging was evaluated using pelvic and bimanual rectal examinations. Tumor diameter was calculated using magnetic resonance imaging (MRI). Metastatic survey was conducted by physical examination, chest radiography, cystoscopy, proctoscopy, and computed tomography (CT).

All patients receive concurrent weekly paclitaxel, carboplatin, and radiation therapy as primary treatment in the present institution. Radiation treatment was administered by external beam pelvic radiotherapy using the four-field box technique (anteroposterior, posteroanterior, and two lateral fields) within one week, approximately, following chemotherapy, when possible. Because the schedule for radiation is typically fully booked, the number of chemotherapy cycles prior to radiation was not restricted to avoid delayed treatment for the cancer patients. A total dose of 45–60 Gy was administered in daily fractions of 1.8–2.0 Gy, five days per week. At 20–30 Gy, a center split was performed. If patients were administered high dose-rate brachytherapy, two to four fractions of intracavitary high dose-rate brachytherapy were administered in weekly fractions of five to six Gy each to point A, based on the external os of the uterus, overlap with the external beam, and tumor volume. The total brachytherapy dose was 12–24 Gy.

The paclitaxel and carboplatin doses were at the treating physician's discretion. Paclitaxel was administered at a weekly dose of 60–70 mg/m^2, with 70 mg/m^2 likely administered to patients with good PS and general condition. Carboplatin was administered based on the area under the curve 2, which is the primary method in the present institute for chemotherapy for cervical cancer [20, 21]. Chemotherapy was administered six to nine times during irradiation or after irradiation; before each cycle, $\geq$ 1,000 neutrophils and $\geq$ 100,000 blood platelets were obtained using growth factors in cases with neutropenia or leukopenia, respectively, at the treating physician's discretion. Patients with hemoglobin levels < 10 g/dL received a red blood cell transfusion before further treatment.

Following completion of the radiation and chemotherapy, patients were examined by cytology, human papillomavirus (HPV) testing using Hybrid Capture 2, CT, and MRI. In cases with a lack of complete response, cytology positive result, or positive HPV test result following the six to none chemotherapy cycles, additional chemotherapy was administered.

Response to treatment, using the RECIST guideline (version 1.1), and toxicity were determined at follow-up evaluations. Post-treatment surveillance was by complete physical examination every month during the first year, every two months for another year (year 2), every three months for another year (year 3), and every six months thereafter. Imaging was obtained by CT every six to 12 months. Acute hematologic and non-hematologic toxicities were recorded based on the Common Toxicity Criteria (CTC) Version 4.0. Acute and late gastrointestinal and genitourinary tract toxicities were recorded using the RTOG/EORTC Late Radiation Morbidity Scoring Criteria.

The present authors used JMP, version 10.0.0 for statistical analyses. Demographic variables are reported as mean ± standard deviation. PFS and OS were analyzed by the Kaplan-Meier method and compared between age, histopathology, stage, PS, and tumor size using log-rank tests because of the short study period. The Cox proportional hazards model was used to adjust for all prognostic factors in multivariable analysis, including survival, stage, tumor histology, PS, and tumor size. For all statistical tests, a p-value < 0.05 was considered significant.

Results

During the study period, 58 patients underwent radiation. Nine patients were excluded because they received only radiation, resulting in a sample size of 49 patients (Figure 1) with a mean age of 57.2 ± 10.5 years (Table 1). One patient was lost to follow-up at 18 months. The basic patient characteristics and prevalence of all stages are shown in Table 1.

There were only eight patients with Stage IVB cervical cancer (Table 1). All but four patients completed their chemotherapy; two patients had grade 4 fatigue, one patient experienced an outbreak of Guillain-Barré syndrome, and one patient experienced a cerebral infraction. An additional patient who experienced a cerebral infraction did not complete the radiation therapy. The total radiation dose was 57.0 ± 8.6 Gy; this included the one patient that did not complete the radiation therapy.

The follow-up lasted a median 32 months (range, four to 75 months). The Kaplan-Meier estimates for PFS and OS

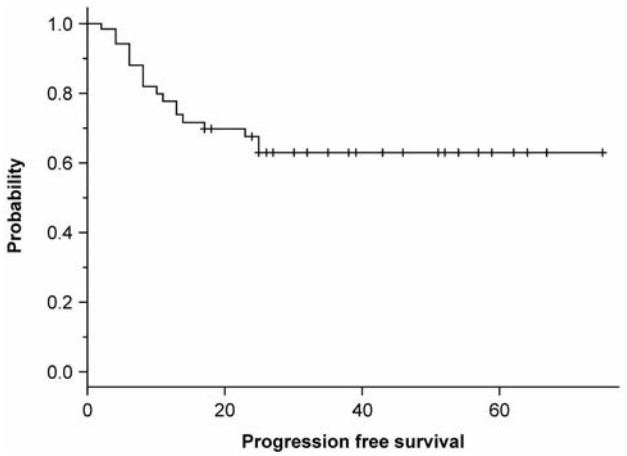

Figure 2. — Kaplan-Meier estimates of progression-free survival in patients with cervical cancer who underwent concurrent chemoradiotherapy with paclitaxel and carboplatin.

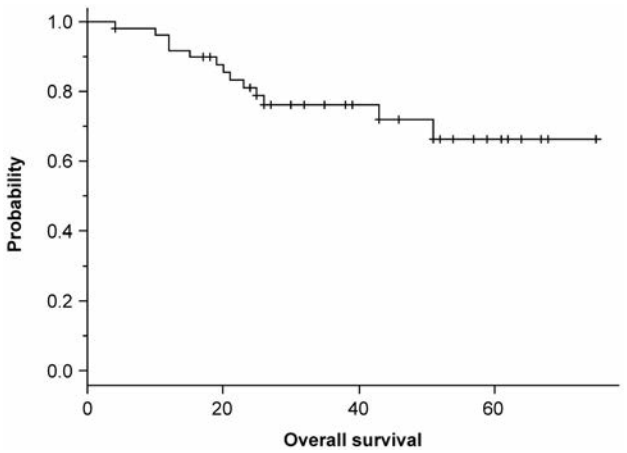

Figure 3. — Kaplan-Meier estimates of overall survival in patients with cervical cancer who underwent concurrent chemoradiotherapy with paclitaxel and carboplatin.

Table 1. — *Demographic and clinical characteristics of patients with Stage IB2-IVB cervical cancer.*

	Total sample (n = 49)
Age (years) (mean ± SD)	57.2 ± 10.5
Tumor size (cm) (mean ± SD)	60.0 ± 19.2 (range, 22–125)
Stage, n (adenocarcinoma)	49 (6)
IB2	8 (1)
IIA1	0
IIA2	4 (0)
IIB	14 (1)
IIIA	5 (2)
IIIB	7 (0)
IVA	3 (0)
IVB	8 (2)
Lymph node, n	
Positive	17
Negative	32
RALS, n	
Yes	28
No	21

RALS: remote afterloading system.

Table 2. — *Response rate to concurrent chemoradiotherapy with paclitaxel and carboplatin in patients with Stage IB2-IVB cervical cancer.*

Stage	n	CR+PR	CR	PR	SD	PD
IB2-IIB	24	100	22 (91.7)	2 (8.3)	0	0
IB2-IIB adenocarcinoma	2	100	1 (50)	1 (50)	0	0
IIIA-IVA SCC	13	92.3	9 (69.2)	3 (23.1)	0	1 (7.7)
IIIA-IVA adenocarcinoma	2	100	1 (50)	1 (50)	0	0
IVB SCC	6	83.3	3 (50)	1 (16.7)	0	2 (33.3)
IVB adenocarcinoma	2	100	1 (50)	0	0	1 (50)
Total	49	91.8	37 (75.5)	8 (16.3)	0	4 (8.2)

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; SCC: squamous cell carcinoma.

are shown in Figures 2 and 3, respectively; the two-year PFS and OS rates were 67.2% and 80.9%. The estimated median PFS and OS rates were 55.1 months and 92.1 months, respectively. Of the patients with a complete or partial response (Table 2), 31.1% (14/45) of the patients experienced recurrence (n = 3, local; n = 9, distant; and n = 2, both local and distant). The distant metastases sites included para-aortic lymph nodes (35.7%, 5/14), lungs (28.6%, 4/14), and the liver (14.3%, 2/14).

In the univariable analysis, histology, PS, tumor size, and age were significant (Table 3); however, only histology was significant in the multivariable analysis (hazard ratio, 6.69; $p = 0.0271$) (Table 4). The PFS was 72% for SCC and 33% for adenocarcinoma.

Acute toxicity grade 3 or 4 neutropenia, anemia, and diarrhea were detected in 85.7%, 8.2%, and 32.7% of the patients, respectively (Table 5). Late toxicity grade 3 or 4 was detected in 12.2% of the patients. Vaginal fistula occurred in three patients, and perforation of the sigmoid colon occurred in one patient; all of these patients had a PS of 3. One patient developed septic shock, but she was treated with antibiotics and recovered.

Discussion

In the present study, treatment of cervical cancer with CCRT, including paclitaxel and carboplatin, was satisfactory, with similar response, PFS, and OS rates to those of previous studies (Table 6) [2, 4, 22], even in patients with

Table 3. — *Relationships between prognostic factors and cervical cancer patient survival in univariable analyses.*

	PFS	*p*	OS	*p*
Stage		0.0096		0.0017
IB2-IIB	84.6		87.8	
IIIA-IVA	53.3		85.1	
IVB	37.5		37.5	
Tumor histology		0.0935		0.0061
SCC	71.9		85.6	
AdenoCa	33.3		33.3	
PS		< 0.0001		0.0006
1	78.8		86.2	
2	40		60	
3	0		60	
Tumor size				
≤ 6 cm	76.7		89.5	0.0371
> 6 cm	52.6	0.013	66.9	
Age (years)		0.654		0.0372
≤ 60	79.2		89.3	
> 60	59		68.2	
Intracavitary therapy				
Yes	82.1	0.008	88.7	
No	47.6		70.2	0.0649

PFS: progression-free survival; OS: overall survival;
SCC: squamous cell carcinoma; AdenoCa: adenocarcinoma;
PS: Eastern Cooperative Oncology Group performance status.

Table 4. — *Relationships between prognostic factors and cervical cancer patient survival in multivariable analysis*

		Hazard radio	95% CI	*p*
Stage	IB2-IIB	1		
	IIIA-IVA	1.51	0.19–10.88	0.68
	IVB	9.93	0.93–129.38	0.0579
Tumor histology	SCC	1		
	AdenoCa	6.69	1.35–35.08	0.0271
PS	1	1		
	2	5.52	0.69–35.64	0.0994
	3	5.34	0.90–38.90	0.0661
Tumor size	≤ 6 cm	1		
	> 6 cm	1.93	0.43–8.05	0.3733
Age (years)	≤ 60	1		
	> 60	3.04	0.66–15.31	0.15
Intracavitary therapy	No	1		
	Yes	0.97	0.11–9.51	0.9784

CI, confidence interval; SCC, squamous cell carcinoma; AdenoCa, adenocarcinoma; PS, Eastern Cooperative Oncology Group performance status.

Table 5. — *Incidence and types of acute and late complications in patients with cervical cancer who underwent concurrent chemoradiotherapy with paclitaxel and carboplatin.*

	Grade				
	0	1	2	3	4
Acute					
Hematologic (neutropenia)	2	3	9	31	4
Hematologic (anemia)	3	22	20	4	0
Thrombocytopenia	42	6	1	0	0
Non-hematologic (vomiting)	36	8	4	0	1
Non-hematologic (diarrhea)	1	19	13	13	3
Late					
Urogenital disorder	34	3	11	1	0
Gastrointestinal disorder	42	0	5	1	1
Lymphedema	49	0	0	0	0
Neuropathy	34	11	1	3	0

Table 6. — *Progression-free survival and overall survival rates reported in previous studies of the use of concurrent chemoradiotherapy with paclitaxel and carboplatin in cervical cancer.*

Reference	Progression-free survival	Overall survival
Keys, *et al.* (1999) [2]	79	85
Eifel, *et al.* (2004) [4]	Not available	73
Whitney, *et al.* (1999) [22]	57	55

Stage III-IV cancer [4]. Regarding adverse effects, neutropenia tended to occur more frequently than previously reported, while gastrointestinal effects were less frequent [2,4,5,22]. Weekly administration of paclitaxel and carboplatin might be tolerable and effective in patients with stage IB2-IVB cervical cancer.

Similar to the results of the present study, a combination of paclitaxel and carboplatin has been reported to be effective chemotherapy [12, 13] as well as acting as a radiosensitizer. A previous *in vitro* study demonstrated an additive effect with concomitant paclitaxel and radiation for SCC [6]. In addition, a phase I study of weekly paclitaxel and carboplatin with concurrent radiotherapy demonstrated similar PFS and OS to those of cisplatin [23]. Furthermore, another member of the taxane family, docetaxel, enhances the efficacy of antivascular therapy when administered weekly; in addition, it confers metronomic chemotherapeutic effects [24]. Therefore, the present treatment may also function as antivascular therapy.

Carboplatin can be administered to patients with severe renal insufficiency [25] and demonstrates lower nephrotoxicity and emetogenicity than cisplatin [9]. Given the relative frequency of neutropenia and gastrointestinal effects in the present study, the authors believe that the regimen they utilized is suitable for outpatients, without requiring hospitalization.

PS and chemotherapy have been reported as independent prognostic factors for survival [26], and there was a tendency in the present study for PS to be a prognostic factor for survival. With a good PS, CCRT can be considered. The combination of taxane and platinum may extend PFS without affecting quality of life [27]. Furthermore, the therapeutic effects of weekly paclitaxel and carboplatin are similar to those of cisplatin [28, 29]. Stage IVB cancer tended to be related with poor survival

outcomes in the present study; however, Stage IIIA-IVA patients may benefit from this CCRT regimen. All of the patients with recurring Stage IVB cancer died, but patients with Stage IIIA-IVA cancer survived with additional treatment. The cancer in these stages (IIIA-IVA) invades locally, while cancers of higher stages spread principally through the lymphatic system; therefore, chemotherapy may be important in these patients [30], and the present regimen may be useful for treating Stage IVB cervical cancer [26].

Adverse effects included bone marrow suppression, with particularly high rates of neutropenia in the present study. Because the data are retrospective and from clinical practice instead of phase I study, the doses chosen by the physicians might not reflect the optimal doses; it is possible that the doses were too high, resulting in toxicity. The outcomes of a phase I trial were published after these patients were treated [23]. In addition, 34.7% (17/49) of the patients were older than 60 years, and the condition of the patients was particularly poor, with 85.7% (42/49) of the patients experiencing at least grade 3 neutropenia and 16.7% (7/49) of the patients with a PS of 2 or 3. Therefore, future clinical trials are needed to determine the optimal dose to avoid neutropenia and bone marrow suppression.

This study has certain limitations. First, because the present study was retrospective in nature, randomized controlled trials should be conducted to reduce potential selection bias in determining PFS and OS. Strict and appropriate protocols should be followed to evaluate adverse effects. Because of the small number of patients with Stage IVB cancer, the results might not generalize to patients with more advanced cancers, and further study should be conducted to gather data in these patients.

The present results indicate that weekly administration of paclitaxel and carboplatin as part of CCRT might be effective for the treatment of cervical cancer. The response, PFS, and OS rates were acceptable, and there were less frequent adverse gastrointestinal effects than previously reported. Future studies should be conducted to compare the efficacy of cisplatin alone with paclitaxel/carboplatin as part of CCRT for the treatment of cervical cancer.

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Detection of high-risk human papillomavirus DNA and immunohistochemical expressions of p16, vimentin, ER, and PR in primary endocervical and endometrial adenocarcinomas

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Summary

Objective: The aim of this study was to explore a panel of useful markers in differential diagnosis of primary endocervical adenocarcinoma (ECA) and endometrial adenocarcinoma (EMA). *Materials and Methods:* Thirty-three ECAs and 31 EMAs were collected and examined for high-risk human papillomavirus (hr-HPV) (16/18) DNA using in situ hybridization, and for p16, vimentin, ER, PR expression using immunohistochemistry (IHC). *Results:* Detection rate of hr-HPV (16/18) DNA in ECA (72.7%, 24/33) was significantly higher than that in EMA (12.9%, 4/31) ($p < 0.01$). Twenty-four of 33 (72.7%) cases of ECA, but only five of 31 (16.1%) cases of EMA showed high expression of p16. Twenty-three of 24 (95.8%) hr-HPV DNA-positive ECA and all four (100.0%) hr-HPV DNA-positive EMA showed high levels of p16 expression. High expression rates of vimentin (90.3%, 28/31), ER (58.1%, 18/31), and PR (71.0%, 22/31) in EMA were significantly higher than those in ECA, respectively ($p < 0.01$). *Conclusion:* Detection of hr-HPV DNA combined with immunohistochemical expressions of p16, vimentin, ER, and PR have important value in differential diagnosis between ECA and EMA.

Key words: hr-HPV DNA; p16; Vimentin; ER/PR; In situ hybridization; Immunohistochemistry; Endocervical adenocarcinoma; Endometrial adenocarcinoma.

Introduction

Endocervical adenocarcinoma (ECA) accounts for 15%-25% of all cervical cancers and the incidence appears to be increasing. Most ECA exhibit a hybrid of endometrioid and mucinous features, or even a dominant or pure endometrioid or mucinous differentiation, therefore ECA and endometrial adenocarcinoma (EMA), share many common morphological characteristics. Only when adenocarcinoma is alone confined to the cervix or the corpus (usually lower uterine segment), the primary ECA or EMA are readily diagnosed. When both the corpus and cervix are involved and precursor lesions are lacking, determining the primary site of a uterine adenocarcinoma can be problematic in hysterectomy specimens [1]. Many recent publications indicated that most ECA are high-risk human papillomavirus (hr-HPV)-related tumors, and diffuse/strong p16 expression can be regarded as a surrogate marker of the presence of hr-HPV [1, 2]. In contrast, most EMA are considered etiologically unrelated to HPV infection, and usually show high levels of vimentin, ER/PR expressions [3-7]. Therefore it is entirely possible to complete the morphological differential diagnosis between ECA and EMA. In the present study, the authors investigated the detection of hr-HPV (16/18) DNA by in situ hybridization (ISH) and expressions of p16, vimentin, ER, and PR by immunohistochemistry (IHC). Their aim was to explore a panel of useful markers in differential diagnosis of primary ECA and EMA.

Materials and Methods

Tissue samples

Thirty-three ECA (22 usual endocervical type, three endometrioid type, three villoglandular type, two intestinal type, two serous type, one adenosquamous carcinoma) and 31 EMA (27 endometrioid type, four serous type) were collected for this study from archives (2010-2012) of the Department of Pathology, Zhongnan Hospital of Wuhan University. The average age of the patients with ECA was 43.8 years (from 27 to 64), and 53.6 years for patients with EMA (from 28 to 80).

Detection of HPV DNA

ISH for hr-HPV (16/18) DNA was performed in all the cases. The hr-HPV (type 16/18) ISH detection kit was utilized. The procedures included slice processing, dewaxing and hydration, microwave heating, hybridization, signal amplification, and chromogenic development [7, 8]. Controls included positive tissue sections of cervical squamous cell carcinoma and negative tissue sections of EMA for hr-HPV (16/18) DNA detection. Cases with brown precipitation or a discrete punctate reaction product (when the copies of viruses were low) in the nucleus were interpreted as positive.

IHC

The expressions of p16, vimentin, ER, and PR were determined according to the manufacturer's instruction. Brown staining in cyto-

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plasm/ nucleus (p16), cytoplasm (vimentin) and nucleus (ER, PR) was accepted as presence of immunoreactivity. The percentage of positive cells was assessed under ×400 microscopy. The expression was classified as following grades: (1) -, if none were stained positive or positive staining cell < 5%; (2) 1+, positive cell occupied 6%-25%; (3) 2+, 26%-50%; (4) 3+,>50%. - and 1+ were categorized as low expression, and 2+ and 3+ were scored as high (over) expression [9]. The cervical squamous cell carcinoma, fibrosarcoma and breast carcinoma tissue section was used as positive control for p16, vimentin, and ER/PR, respectively. The PBS solution was used to replace the primary antibody as negative control.

Statistical analysis

The SPSS 11.0 statistics program was used for data analysis. The chi-square test was used and $p < 0.05$ was accepted as statistically significant.

Results

The detection of HPV (16/18) DNA in ECA and EMA

A total of 24 of the 33 (72.7%) ECA were found to be hr-HPV (16/18) DNA-positive by ISH, but the difference of detection rate for hr-HPV (16/18) DNA was not significant in histological subtypes ($p > 0.05$). Only four of 31(12.9%) EMA were hr-HPV (16/18) DNA-positive, but detection rate of hr-HPV (16/18) DNA in serous type (75.0%, 3/4) was significantly higher than that of endometrioid type (1/27, 3.7%) ($p < 0.01$). Statistically, detection rate of hr-HPV (16/18) DNA in ECA was significantly higher than that in EMA ($p < 0.01$) (Table 1) (Figures 1A, 2A).

Table 1. — *Detection of hr-HPV (16/18) DNA and expression of p16 in ECA and EMA (n, %).*

Group	n	hr-HPV DNA (+)	p16 expression* Low	High
Cervical adenocarcinoma	33	24 (72.7%)	9 (27.3)	24 (72.7)
endocervical type	22	17 (77.3%)	6 (27.3)	16 (72.7)
villoglandular type	3	2 (66.7%)	1 (33.3)	2 (66.7)
endometrioid type	3	2 (66.7%)	0 (0.0)	3 (100.0)
intestinal type	2	2 (100.0%)	0 (0.0)	2 (100.0)
serous type	2	1 (50.0%)	1 (50.0)	1 (50.0)
andenosquamous carcinoma	1	0 (0.0%)	1 (100.0)	0 (0.0)
Endometrial adenocarcinoma	31	4 (12.9%)	26 (83.9)	5 (16.1)
endometrioid type	27	1 (3.7%)	25 (92.6)	2 (7.4)
serous type	4	3 (75.0%)	1 (25.0)	3 (75.0)
Total	64	28 (43.8%)	35 (54.7)	29 (45.3)

* - and 1+ were categorized as low expression;
2+ and 3+ were scored as high expression.

The expression of p16 and correlation between hr-HPV DNA detection and expression of p16 in ECA and EMA

Twenty-four of 33 (72.7%) cases showed high expression of p16 in ECA, but only five of 31 (16.1%) cases in EMA exhibited high expression of p16. The difference was significant between the former and the latter ($p < 0.01$).

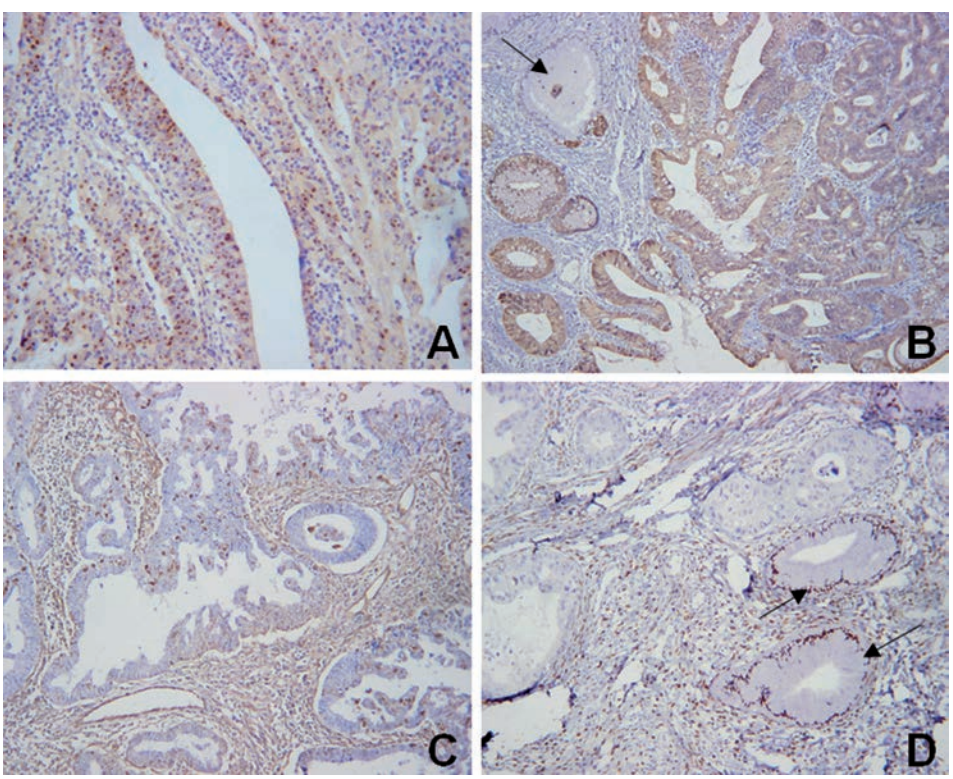

Figure 1. — Detection and expression of hr-HPV (16/18) DNA, p16, vimentin, and ER in ECA. (A) The hr-HPV (16/18) DNA detection demonstrate brown punctate positive reaction product within tumor cell nuclei of ECA. In situ hybridization ×20. (B) The p16 staining shows diffuse cytoplasmic and nuclear expression in case of hr-HPV DNA (+) ECA. Normal endocervical glands (black arrow) exhibit negative staining (×10). (C) The vimentin staining shows negative expression in ECA. The endometrial stroma exhibits positive staining (×10). (D) The ER staining shows negative expression in ECA. The endometrial stroma and residual endocervical glands (black arrows) are visualized as positive control (×10).

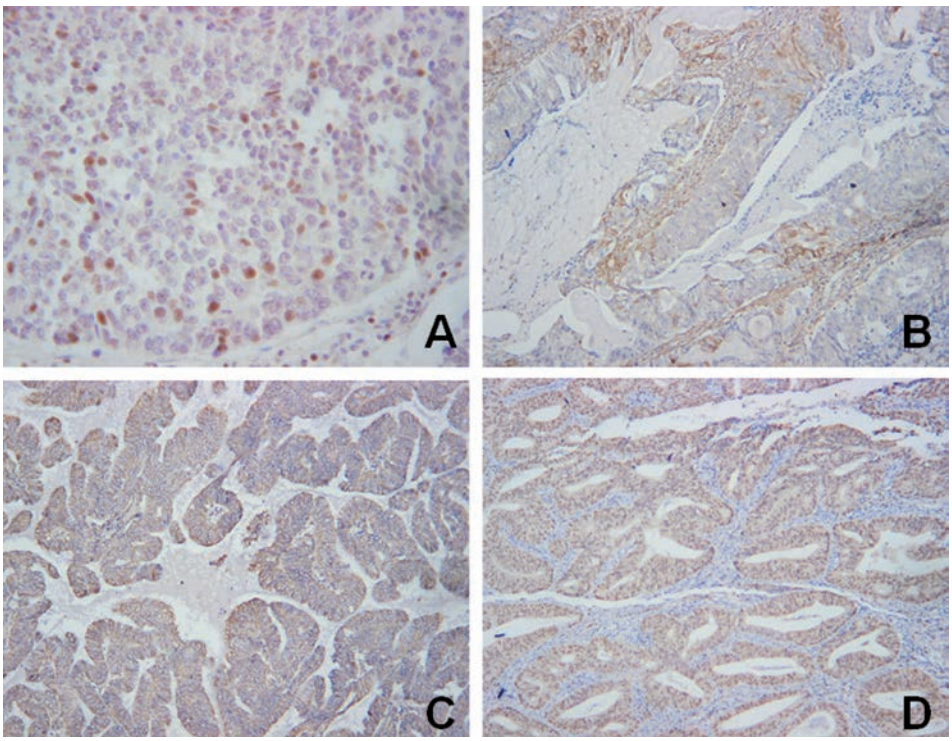

Figure 2. — Detection and expression of hr-HPV (16/18) DNA, p16, vimentin, and PR in EMA. (A) The hr-HPV (16/18) DNA detection demonstrates brown positive precipitation within tumor cell nuclei of some EMA. In situ hybridization ×40. (B) The p16 protein staining shows focal positive cytoplasmic and nuclear expression in case for hr-HPV DNA(-) EMA (×10). (C) The vimentin staining shows diffuse cytoplasmic expression in EMA (×10). (D) The PR staining shows diffuse positive expression within tumor cell nuclei of endometrial adenocarcinoma (×10).

Table 2. — *Correlation between hr-HPV(16/18) infection and p16 high expression in ECA and EMA.*

Group	hr-HPV (16/18) DNA detection (n)	p16 expression high (n)	X^2	*p*
ECA	+, 24	23	5.13	< 0.05
	-, 9	1		
EMA	+, 4	4	11.2	< 0.01
	-, 27	1		

High levels of p16 expression did not correlate with various histological subtypes of ECA ($p > 0.05$), but the rate of p16 high expression in serous type (75.0%, 3/4) was higher than that in endometrioid type (7.4%, 2/27) of EMA ($p < 0.01$) (Table 1) (Figures 1B, 2B).

Twenty-three of 24 (95.8%) hr-HPV DNA-positive ECA cases showed high expression of p16, but only 1 of 9 (11.1%) hr-HPV DNA-negative ECA cases showed p16 high expression. All 4 (100.0%) hr-HPV DNA-positive EMA cases presented p16 high expression, but 1 of 27 (3.7%) of hr-HPV DNA-negative EMA cases exhibited p16 high expression. Regarding the incidence of p16 high-level expression, there was significant difference between hr-HPV DNA-positive and -negative cases of ECA ($p< 0.05$) and EMA ($p< 0.01$), respectively (Table 2) (Figure 1B, 2B).

The expression of vimentin, ER, and PR in ECA and EMA

All 33 cases of ECA showed low expression of vimentin (no detection in 32 cases, weak positive expression in one case). Twenty-eight of 31 (90.3%) EMA cases presented high levels of vimentin expression, but difference for high levels of vimentin expression was significant between endometrioid (96.3%, 26/27) and serous type (50.0%, 2/4) of EMA ($p < 0.01$). Statistically, the rate of high expression of vimentin in EMA was higher than that in ECA, and the difference was dramatic ($p < 0.01$) (Table 3) (Figure 1C, 2C).

All 33 cases of ECA showed low expression of ER (no detection in 32 cases and minimal positive expression in one case). ER High expression rate was 58.1% (18/31) in EMA, but high expression rate in endometrioid type (18/27, 66.7%) was higher than that of serous type (0/4, 0.0%) ($p < 0.05$). The high expression rate of ER in EMA was evidently higher than that in ECA ($p < 0.01$) (Table 3) (Figure 1D).

All 33 cases of ECA exhibited low expression (negative staining) of PR. High expression rate of PR was 71.0% (22/31) in EMA, but difference of high expression rate between endometrioid type (81.5%) and serous type (0.0%) was significance ($p < 0.01$). Statistically, the high expression rate of PR in EMA was significantly higher than that in ECA ($p < 0.01$) (Table 3) (Figure 2D).

Discussion

Infection of hr-HPV is the main causative event in the development of cervical cancer and hr-HPVs have been detected

Table 3. — *Expressions of vimentin, ER, and PR in endocervical and endometrial adenocarcinoma (n, %).*

Group	n	Vimentin expression*		ER expression*		PR expression*	
		Low	High	Low	High	Low	High
Cervical adenocarcinoma	33	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)
endocervical type	22	22 (100.0)	0 (0.0)	22 (100.0)	0 (0.0)	22 (100.0)	0 (0.0)
villoglandular type	3	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)
endometrioid type	3	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)
intestinal type	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
serous type	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
andenosquamous carcinoma	1	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
Endometrial adenocarcinoma	31	3 (9.7)	28 (90.3)	13 (41.9)	18 (58.1)	9 (29.0)	22 (71.0)
endometrioid type	27	1 (3.7)	26 (96.3)	9 (33.3)	18 (66.7)	5 (18.5)	22 (81.5)
serous type	4	2 (50.0)	2 (50.0)	4 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)
Total	64	36 (56.3)	28 (43.7)	46 (71.9)	18 (28.1)	42 (65.6)	22 (34.4)

* –and 1+ were categorized as low expression; 2+ and 3+ were scored as high expression.

in up to 99.7% of squamous cell carcinoma [10]. At present, although infection frequency and viral type of hr-HPV are distinct in many studies, most ECAs (~90.0%) have been regarded as hr-HPV-related tumors. In contrast, infection of hr-HPV is rarely seen in EMA. Plunkeff *et al.* found that 78.0% (39 of 50) ECAs contained hr-HPV DNA, compared with 2.0% (1 of 50) EMAs that was positive for hr-HPV DNA [6]. Hadzisejdć *et al.* reported that 81 out of 89 (91.0%) tested positive for hr-HPV DNA in ECA, in which, statistically significant predominance of single hr-HPV (type 18) infections in adenocarcinoma in situ (AIS) and endocervical adenocarcinoma (AC) whereas multiple hr-HPV (16/18 type) infections were more abundant in AC compared with AIS [8]. In cervical cancer associated with hr-HPV infection, functional inactivation of Rb by HPV E7 protein results in an accumulation of p16 protein because normally Rb inhibits transcription of p16 [10,11]. As a consequence, most ECA exhibit a diffuse positivity of p16, whereas EMA (especially endometrioid type) is usually negative or there is focal positivity [3, 5, 12, 13]. Expression of p16 protein is associated with HPV oncogenic potential in cervical and genital lesions [14]. Thus, p16 protein is also a useful marker to help determine the tumor origin such as ECA and EMA (endometrioid type). In the present study, detection rate of hr-HPV (16/18) DNA was 72.7% (24/33) in ECA, compared with 12.9% (4/31) in EMA. High p16 expression rate of ECA (72.7%, 24/33) was significantly higher than that of EMA (16.1%, 5/31). The authors also found that the rate of p16 high expression was 95.8% (23/24) in hr-HPV-positive cases of ECA, and all four (100.0%) hr-HPV DNA-positive EMA presented p16 high expression. The present results and other studies suggest that hr-HPV DNA detection and p16 expression level could be a useful adjunct to distinguish between ECA and EMA. There have been some controversial reports regarding the role of the p16 protein in the differential diagnosis of ECA and EMA. Recently, Saad *et al.* proposed that the expression of p16 in undifferentiated carcinoma of the uterus does not exclude its endometrial origin. They found that diffuse/strong positive staining for p16 was seen in 40/50 (80%) cases of ECA and 14/28 (50%) cases of undifferentiated endometrial carcinoma. At present, distinguishing between undifferentiated endometrial carcinoma and endocervical adenocarcinoma, both of which share diffuse p16 expression, should rely on simultaneous detection of human papilloma virus in the latter [15]. Because most ECAs are hr-HPV-related tumors and the diffuse p16 positivity can be regarded as a surrogate marker of the presence of hr-HPV, p16 marker is currently the most important focus of attention and has been most widely applied in the field of gynecologic pathology [16].

Vimentin is an intermediate filament protein normally expressed in mesenchymal cells, but the aberrant expression of vimentin can be found in epithelial cancer cells [17]. For a long time, vimentin is a useful and reliable marker to identify the primary ECA and EMA (especially endometrioid type). Azumi *et al.* have indicated that as a diagnostic reagent, antibodies to vimentin are of the greatest application in the diagnosis of carcinoma of uncertain primary site, whereas strong co-expression of vimentin and keratin may be a clue to renal, endometrial, and thyroid carcinomas [18]. Many studies showed that the positive expression rates for vimentin were significantly different between primary ECA and EMA [19]. The positive expression rate of vimentin in the former was approximately 7%-14% compared with 62%-93% in the latter [20-22]. In the present study, 32 cases did not have any expression of vimentin and only one case was weak positive expression in 33 cases of ECA. In contrast, the high expression rate for vimentin was 90.3% (28/31) in EMA, especially in EMA of endometrioid type with high expression rate of 96.3% (26/27). The present research results strongly suggest that vimentin is indeed a valuable marker in the differential diagnosis of ECA and EMA.

The endometrium is one principal target tissue of the pituitary-gonadal axis, but has also been recognized as an endocrine organ. Human endometrium expresses ER and PR, which are related to autocrine and paracrine processes that respond to estrogen and progesterone. The ER and PR expres-

sion and distribution pattern may play an important role in endometrial function and pathogenesis [23]. Therefore, EMA (especially endometrioid type) is typically hormone-dependent. Many studies reported that the EMA (endometrioid type) significantly expresses ER and PR. In contrast, ECA usually have absent or limited expression of ER and PR [1, 7, 22-24]. According to literature, positive expression rates of ER and PR in EMA were 67% - 97% and 89% - 96%, compared with 4% - 20%, and 4% - 21% in ECA, respectively [20-22]. The present results show that the high expression rates of ER and PR were 58.1% and 71.0%, respectively, in the EMA, and 66.7% and 81.5%, especially in the endometrioid type of EMA. However, ER and PR expressions were low in ECA (the vast majority did not express), with no high-expression cases. The high expression rates of ER and PR in EMA was evidently higher than that in ECA ($p < 0.01$). The present results were consistent with what have been reported in the literature.

The present study demonstrated once again that the detection of hr-HPV DNA combined with immunohistochemical expressions of p16, vimentin, ER, and PR are useful for differential diagnosis between ECA and EMA and clarifying the origin of uterine adenocarcinomas.

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Comparison of p57, c-erbB-2, CD117, and Bcl-2 expression in the differential diagnosis of hydatidiform mole and hydropic abortion

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Summary

Purpose: To explore the utility of p57, c-erbB-2, CD117, and Bcl-2 immunostaining in the differential diagnosis of complete hydatidiform mole (CHM), partial hydatidiform mole (PHM), and hydropic abortion (HA). *Materials and Methods:* Immunohistochemical expression of the p57, c-erbB-2, CD117, and Bcl-2 proteins were investigated semi-quantitatively using paraffin-embedded tissue sections from histologically unequivocal cases of CHM (n = 20), PHM (n = 23), and HA (n = 17). *Results:* All cases of CHM exhibited a striking absence of p57 expression. The percentage of positive p57 staining was similar between PHMs (73.9%) and HAs (76.5%) ($p > 0.05$). The comparison of c-erbB-2 expression revealed a significantly higher percentage of positive c-erbB-2 staining in CHMs (45%) compared with that in PHMs (8.7%) and HAs (5.9%) ($p = 0.006$ and 0.01, respectively). The CD117 expression pattern (immunoreactivity score, percentage of positive cells, and staining intensity) was significantly lower in HAs compared with that in PHMs and CHMs ($p < 0.05$ for all). A significantly increased Bcl-2 expression pattern was observed in HAs compared with that in PHMs and CHMs ($p < 0.05$ for all). *Conclusion:* Immunohistochemical examination of p57, c-erbB-2, CD117, and Bcl-2 expression represents a relatively simple, reliable, and cost-efficient procedure to definitively distinguish among CHM, PHM, and HA.

Key words: Bcl-2; CD117; c-erbB-2; Hydatidiform mole; p57.

Introduction

Hydatidiform moles (HMs) are the most common form of gestational trophoblastic disease (GTD) resulting from abnormal fertilisation and are characterised by hydropic swelling of placental villi and trophoblastic hyperplasia. They are categorised into two distinct entities of partial hydatidiform mole (PHM) and complete hydatidiform mole (CHM), based on morphological, genetic, and clinical features. In addition, hydropic abortions (HAs) can mimic HMs morphologically. Accurately distinguishing HAs from HMs and PHMs from CHMs is important for appropriate clinical management, as the risk of persistent GTD is higher in patients with CHM (10–30%) than with PHM (0.5–5%), whereas HA is completely benign and not associated with the risk of persistent GTD [1]. A diagnosis of HM can often be made based on a morphological assessment alone. However, there are significant overlaps in the histological features between HMs and HAs, as well as between CHMs and PHMs, causing considerable inter-observer and intra-observer variability in the diagnosis [2].

Genomic imprinting (gene expression based on gametes of origin) is important for the regulation of implantation and embryonic development. CHMs are derived exclusively from the paternal genome (androgenetic diploidy), whereas PHMs contain one maternally derived and two paternally derived haploid genomes (diandric triploidy), suggesting that both CHM and PHM are the result of abnormal expression of imprinted genes. The p57 gene is paternally imprinted and expressed predominantly from the maternal allele in most tissues. p57 is a potent cell cycle inhibitor and tumour suppressor, and lack of p57 expression in trophoblastic disease plays a relevant role in its abnormal proliferation and differentiation [3].

The c-erbB-2 (also known as Her-2/neu) protein is a transmembrane tyrosine kinase receptor in the epidermal growth factor receptor family. This proto-oncogene is involved in activating pathways leading to cell growth and differentiation. The c-erbB-2 protein is expressed frequently at low levels in a variety of adult epithelial cells; however, aberrant activation of c-erbB-2 due to amplification and/or overexpression can contribute to unrestrained proliferation and tumour development [4].

CD117, also known as c-kit, is a tyrosine kinase receptor that regulates cell proliferation, apoptosis, adhesion and chemotaxis. CD117 may be involved in proliferation and normal differentiation of the placenta during pregnancy

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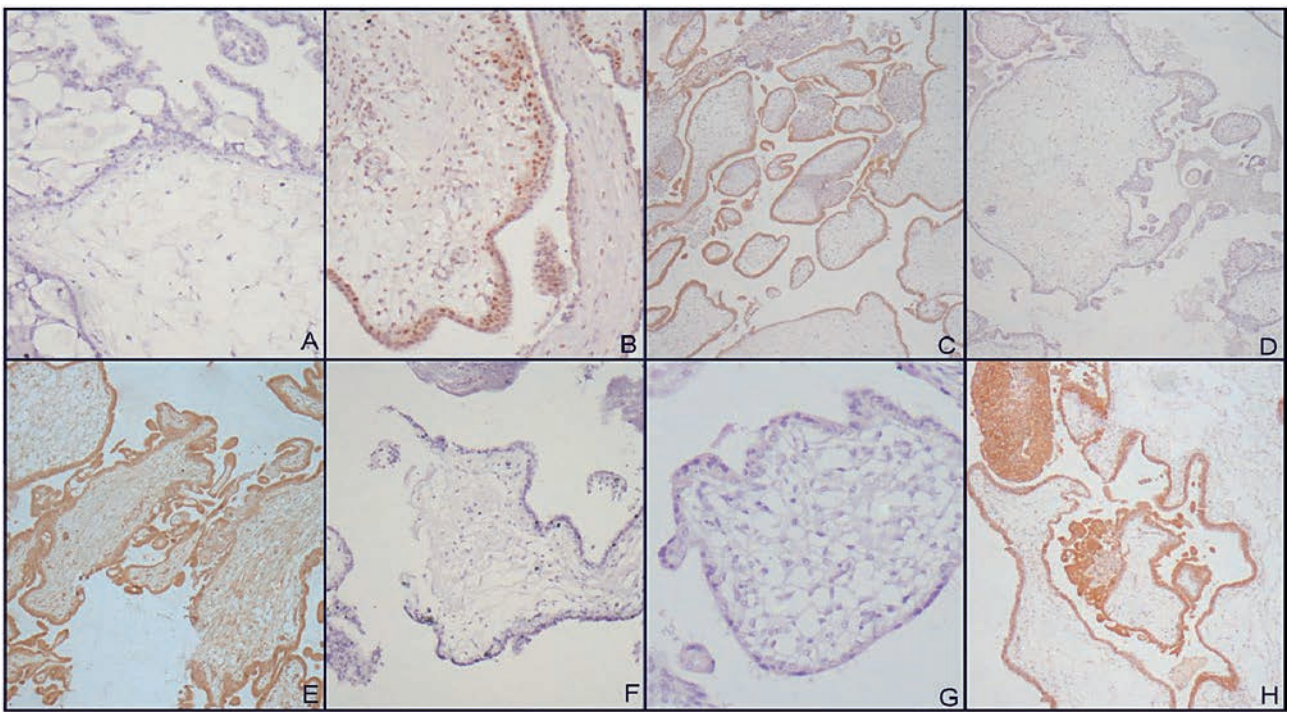

Figure 1. — A) Villi from a complete hydatidiform mole (CHM) demonstrating complete loss of p57 immunostaining of both the villous cytotrophoblast and stromal cells (magnification, ×100). B) Villi from a partial hydatidiform mole (PHM) demonstrating positive p57 immunostaining of both the villous cytotrophoblast and stromal cells (magnification, ×100). C) Bcl-2 protein cytoplasmic staining (strong intensity) is seen in the synctiotrophoblastic and cytrophoblastic cells of PHM (magnification, ×40). D) Lack of Bcl-2 staining is seen in the CHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×40). E) CD117 cytoplasmic staining (strong intensity) is seen in the CHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×100). F) Lack of CD117 staining is seen in PHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×40). G) Negative c-erbB-2 immunostaining in hydropic abortion (magnification, ×200). H) Positive c-erbB-2 immunostaining of CHM synctiotrophoblastic and cytotrophoblastic cells (magnification, ×40).

[5]. Overexpression of CD117 has been implicated in the pathogenesis of numerous tumours including choriocarcinoma [6].

Apoptosis plays an important role in normal placental morphogenesis and in the pathogenesis of GTD [7]. Bcl-2, an anti-apoptotic molecule residing in the mitochondria, plays a decisive role regulating cell death during embryogenesis and normal placental growth, and its dysregulation has been implicated in several pregnancy disorders [8].

The objectives of this study were to ascertain the expression patterns of p57, c-erbB-2, CD117, and Bcl-2 in CHMs, PHMs, and HAs, and to assess the value of these markers in the differential diagnosis of the three entities.

Materials and Methods

Case selection

Sixty-seven formalin-fixed paraffin-embedded gestational specimens with hydropic swelling of chorionic villi were retrieved from the files of the Department of Pathology, Antalya Training and Research Hospital between January 2010 and December 2012 after institutional review board approval. The diagnosis of each case (CHM, PHM or HA) was obtained from the original pathology report. Hematoxylin and eosin-stained sections of the specimens were reviewed independently by two pathologists with no knowledge of the specimens' clinical information and classified as CHM, PHM or HA according to the main morphological findings [9]. CHM is characterized by hydropic swelling of villi with central cisterns, circumferential trophoblastic hyperplasia with diffuse and marked atypia, and trophoblastic inclusions. Morphologic features of PHM include focal trophoblastic hyperplasia, a dimorphic villous population with an admixture of hydropic and normal villi, scalloping and prominent stromal trophoblastic inclusions, and mild trophoblastic atypia. HA is characterized by villous edema without trophoblastic hyperplasia. Sixty cases were histologically unequivocal for CHM (n =20), PHM (n = 23), and HA (n = 17) and constituted the study group. The remaining seven equivocal cases were difficult to classify as HA, PHM or CHM because of mixed histological features and were excluded from the final statistical analysis. These equivocal cases were subjected to molecular genotyping. Patient demographic data were obtained through a chart review. Serum beta-human chorionic gonadotropin (β-hCG) levels were measured by a two-site chemiluminescence immunoassay based on the direct sandwich technique. The inter- and intra-assay coefficients of variation were 4.1% and 1.3%, respectively.

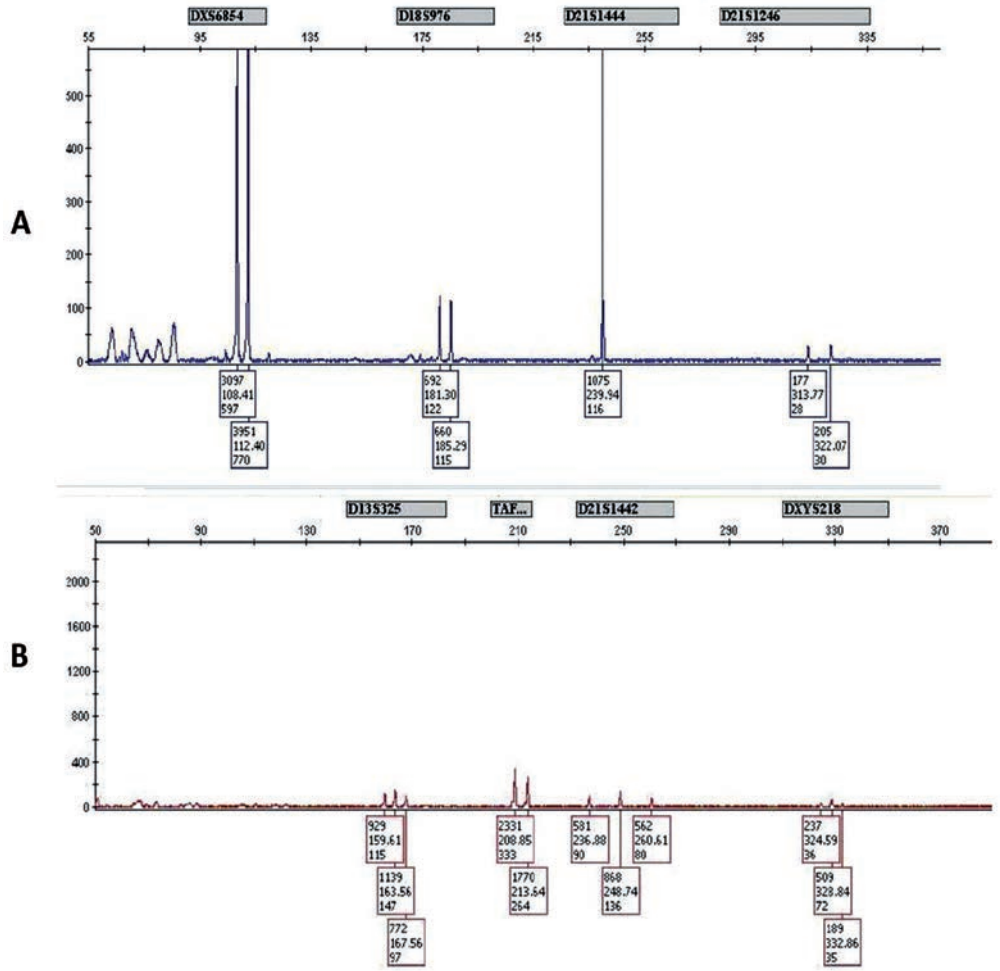

Figure 2. — Representative examples of diploid and triploid histograms produced by short-tandem repeat amplification. A) Three loci (DXS6854, D18S976, and D21S1246) each demonstrate two alleles, consistent with diploidy (two peaks with approximate 1:1 ratios). B) Three loci (D13S325, D21S1442, and DXYS218) each demonstrate three alleles, consistent with triploidy.

Tissue preparation and evaluation of immunohistochemical staining

Briefly, four-µm thick, representative sections from formalin-fixed, paraffin-embedded tissue blocks were obtained in each case, incubated for 120 minutes at 60°C and then overnight at 37°C. The tissue sections were deparaffinzed in xylene and alcohol, rehydrated, washed in a solution buffered with 10% sodium citrate in a microwave oven (800 W). The slides were left to cool at room temperature for 20 minutes. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide, and the slides were washed in phosphate-buffered saline (PBS; ten mM, pH 7.4). As the primary antibody, mouse monoclonal antibodies against p57 (clone 25B2; 1:50 dilution), c-erbB-2 (clone 10A7; 1:40 dilution), CD117 (clone 57A5D8, ready to use), and Bcl-2 (clone 3.1; 1:80 dilution) were incubated with the slides for 60 minutes at room temperature, according to the manufacturer's protocol. The slides were washed in PBS, and the sections were incubated for 20 minutes with biotinylated secondary antibody. The chromogenic reaction was performed using 3,3'diaminobenzidine. Then, the sections were washed in distilled water, counterstained with hematoxylin and mounted with Entellan. Appropriate positive and negative controls were run for each case. The evaluation of protein expression was performed independently by two pathologists (DS and BT). The stained cell types were identified as either a villous cytotrophoblast, villous intermediate trophoblast, villous syncytiotrophoblast, villous stromal cells or decidual cells. All samples were scored semi-quantitatively. Immunohistochemical results were recorded independently of the original clinicopathological diagnosis. Representative case examples are illustrated in Figure 1.

On the basis of the staining pattern reported in the literature, the specimens were interpreted as "positive" for p57 staining when distinct nuclear staining (> 50%) of villous stromal cells and cytotrophoblasts was observed. The p57 stain was interpreted as "negative" when there was no distinct staining or limited nuclear staining (< 10%) of villous stromal cells and cytotrophoblasts but intermediate trophoblasts and/or maternal decidua exhibited nuclear expression of p57 (which served as the positive internal control for all cases). Nuclear expression in villous stromal cells and cytotrophoblasts in the focally positive range (10–50%) was considered an equivocal result [10]. Syncytiotrophoblastic cells were used as negative controls.

c-erbB-2 expression was analysed by evaluation of the staining intensity and the proportion of stained villous trophoblastic cells as follows: (0): no staining or staining observed in < 10% of the villous trophoblast cells, (+): weak staining observed in > 10% of the villous trophoblast cells in part of the cell membrane, (++): weak to moderate complete membrane staining observed in > 10% of the villous trophoblast cells, and (+++): strong complete membrane staining observed in > 10% of the villous trophoblast

Table 1. — *Immunohistochemical analysis of p57, c-erbB-2, CD117, and Bcl-2 in hydropic abortions, partial, and complete hydatidiform moles.*

Marker expression	HA (n=17)	PHM (n=23)	CHM (n=20)	*p*-value	p^1-value	p^2-value	p^3-value
p57 [a]				< 0.001	< 0.001	0.999	< 0.001
Negative	4 (23.5)	6 (26.1)	20 (100)				
Positive	13 (76.5)	17 (73.9)	-				
c-erbB-2 [a]				0.003	0.006	0.999	0.01
Negative	16 (94.1)	21 (91.3)	11 (55.0)				
Positive	1 (5.9)	2 (8.7)	9 (45.0)				
CD117 [b]							
Staining intensity	1.06±0.24	1.78±0.60	2±0.72	< 0.001	0.307	< 0.001	< 0.001
Percentage of positive cells	1.88±0.70	2.65±0.89	2.75±0.91	0.002	0.857	0.005	0.005
Immunoreactivity scores	2.06±1.20	5.09±2.81	6.05±3.87	< 0.001	0.651	0.001	< 0.001
Bcl-2 [b]							
Staining intensity	2.29±0.77	1.61±0.84	0.75±0.55	< 0.001	0.001	0.014	< 0.001
Percentage of positive cells	1.35±0.1	0.72±0.21	0.70±0.47	0.005	0.015	0.009	0.005
Immunoreactivity scores	2.29±0.77	1.61±0.84	0.75±0.55	< 0.001	0.001	0.014	< 0.001

Values are given as [a] number (%) or [b] mean ± SD (standard deviation). If the Kruskal–Wallis test was positive ($p < 0.05$) then post-hoc analysis was applied. *p,* between three groups; p^1, between partial and complete hydatidiform mole; p^2, between partial hydatidiform mole and hydropic abortion; p^3, between complete hydatidiform mole and hydropic abortion. Adjusted significance level for p^1, p^2, and $p^3 = 0.017$.
HA: hydropic abortion, PHM: partial hydatidiform mole, and CHM: complete hydatidiform mole.

cells [11]. c-erbB-2 expression was categorised as positive or negative, and only the score (+++) was considered a positive reaction. Sections of two breast carcinomas known to express c-erbB-2 served as positive controls.

CD117 and Bcl-2 expression in villous cells was evaluated using an immunoreactivity score (IRS) as described elsewhere [12]. The IRS was calculated by multiplying the percentage of positive cells (PP) by the staining intensity (SI). PP was estimated by counting ~ 100 cells per slide (×400 magnification) and scored as follows: 0 = < 5% staining, 1 = 5–25% staining, 2 = 25–50%, 3 = 50–75% staining, and 4 = > 75% staining. The SI was scored as follows: 0: negative, 1: weakly positive, 2: moderately positive and 3: strongly positive. Cytoplasmic staining was the criterion for positive CD117 and Bcl-2 reactions. Sections of a lymph node with follicular hyperplasia were used as a positive control for Bcl-2, and sections of a gastrointestinal stromal tumour were used as a positive control for CD117.

DNA analysis by short tandem repeat (STR) genotyping

DNA extraction was performed on formalin-fixed, paraffin embedded tissue following a standard procedure using an automated system. Quantitative fluorescent polymerase chain reaction (QF-PCR) methodology was used to determine the diploidy status of the extracted DNA. Short-tandem repeat loci were evaluated in each sample using the ChromoQuant QF-PCR kit, which allows for DNA amplification and fluorescence analysis of 22 loci from different chromosomes and the amelogenin locus simultaneously. The amplified microsatellite fragment size data were analysed using ChromoQuant Visualizer STaR ver. 4.03 analysis software. QF-PCR amplification and capillary electrophoresis were performed according to the manufacturer's instructions. Capillary electrophoresis data from villous tissues were analysed to identify alleles at each locus. For each locus from which two alleles were identified, the allelic ratio was calculated by dividing the peak height of the longer allele by the peak height of the shorter allele. Allelic ratios of 0.8–1.4 were considered consistent with diploidy. Allelic ratios < 0.65 or > 1.8 were considered to be consistent with triploidy. Allelic ratios that fell between the normal and abnormal ranges were classed as inconclusive. In addition, loci with three and two alleles identified were consistent with triploidy and diploidy, respectively (Figure 2). At least two informative loci were required for the final interpretation.

Statistical analysis

The comparison of antibodies among histologically unequivocal cases was assessed using SPSS 18.0 software. The numeric variable comparison among the three groups was performed using the Kruskal–Wallis test, and post-hoc comparisons were assessed using the Mann–Whitney *U*-test with Bonferroni's correction. Categorical data were compared using Pearson's chi-square test. All tests were two-sided at a significance level of $p < 0.05$.

Results

The age of the 67 patients ranged from 17 to 45 years (median, 27) with gestational ages of 6–15 weeks (median, ten). Patients with PHM (median, 26 years) were younger than those with CHM (median, 29 years), and HA (median, 31 years). The median gestational age at the time of diagnosis was nine weeks for the HA cases, 11 weeks for PHM cases, and ten weeks for CHM cases. All patients with HMs were followed by clinical examination and serum β-hCG measurements. After therapeutic evacuation, one patient with CHM evolved into persistent trophoblastic disease requiring methotrexate therapy. However, the β-hCG level was negative after six months of therapy. The average follow-up period in cases of HMs was 12.8 months (range 12–14).

The comparison of p57, c-erbB-2, CD117, and Bcl-2 expression in the histologically unequivocal cases is summarised in Table 1. All 20 cases that had been morpho-

Table 2. — *Molecular genotyping results in the histologically undetermined cases.*

Case	Clinical impression	Morphologic impression	p57 staining	Genotyping results	Final diagnosis
1	Missed abortion	HA *vs.* PHM	Positive	Biparental diploidy	HA
2	Missed abortion	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM
3	Incomplete abortion	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM
4	Incomplete abortion	CHM *vs.* PHM	Equivocal	Diandric triploidy	PHM
5	Hydatidiform mole	CHM *vs.* PHM	Equivocal	Diandric triploidy	PHM
6	Hydatidiform mole	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM
7	Hydatidiform mole	CHM *vs.* PHM	Negative	Androgenetic diploidy	CHM

HA, hydropic abortion; PHM, partial hydatidiform mole; CHM, complete hydatidiform mole.

logically diagnosed as CHM exhibited a striking lack of p57 positive staining in villous cytotrophoblasts and stromal cells. Although the percentage of positive p57 staining tended to be higher in HAs (76.5%) than in PHMs (73.9%), the difference was not significant ($p = 0.999$). Maternal decidua and intermediate trophoblasts showed strong p57 expression in contrast to the syncytiotrophoblast, which showed complete negativity in all cases regardless of the diagnosis.

c-erbB-2 expression was observed in all types of villous trophoblasts. The villous stromal cells and decidual cells showed negative immunostaining. A significant between-group difference was observed in the percentage of positive c-erbB-2 staining ($p = 0.003$). Pair-wise comparisons between the groups revealed a significantly higher percentage of positive c-erbB-2 staining in CMs (45%), compared with PHMs (8.7%) and HAs (5.9%) ($p = 0.006$ and 0.01, respectively). However, no significant difference was observed between PHMs and HAs ($p = 0.999$).

Cells positively expressing CD117 were restricted mostly to villous trophoblasts, whereas decidual and villous stromal cells showed weak immunostaining. The expression pattern (SI, PP, and IRS) was significantly lower in HAs compared with PHMs and CMs, as shown in Table 1. Although the expression pattern tended to be higher in CMs than in PHMs, the difference was not significant (adjusted $p > 0.017$).

Bcl-2 expression was observed in all types of villous trophoblasts. Villous stromal cells and decidual cells showed negative immunostaining. A significance between-group difference was observed in the Bcl-2 expression pattern ($p < 0.05$). A pair-wise comparison between the groups revealed significantly increased expression in HAs compared with PHMs and CHMs, and also in PHMs compared with CHMs (adjusted $p < 0.017$ for all)

Seven histologically undetermined cases included one case with a differential diagnosis between HA and PHM, whereas the other six cases had a differential diagnosis between PHM and CM (Table 2). DNA analysis by STR genotyping was performed to refine the histological subtypes of these cases. Case with a differential diagnosis between HA and PHM showed positive p57 expression and cytogenetic diploidy, consistent with a HA. Among the six cases with a differential diagnosis between PHM and CM, two cases showed equivocal results for p57 immunostaining and cytogenetic triploidy, consistent with PHM, and the remaining four cases showed negative p57 immunostaining and cytogenetic diploidy, consistent with CM.

Discussion

Accurate clinical diagnosis is important for distinguishing among CHM, PHM, and HA. A careful microscopic evaluation of the morphological features observed on haematoxylin and eosin-stained slides remains the cornerstone of diagnosis for these three entities. However, classifying HMs based solely on histological appearance can be extremely difficult, even for an experienced pathologist. In addition, HAs may exhibit atypical trophoblast proliferation leading to an erroneous diagnosis of HM. Several ancillary techniques have been applied to resolve these diagnostic problems, including immunohistochemistry, conventional cytogenetics (karyotyping), flow cytometry, digital image analysis, fluorescence *in situ* hybridisation (FISH), and molecular genotyping [13].

The value of an immunohistochemical analysis of the paternally imprinted, maternally expressed p57 gene for improving the diagnosis of HMs has been well established. p57 is a highly specific and sensitive marker for CHM due to an absence of nuclear staining in villous stromal cells and cytotrophoblasts [14-16]. The lack of p57 activity in CHM cases can lead to loss of cell cycle control, resulting in abnormal proliferation and differentiation of trophoblasts, correlated with histological features such as trophoblastic hyperplasia. In contrast, both PHMs and HAs contain a maternal chromosomal complement and express p57. This differential p57 staining pattern is helpful for distinguishing CHMs from PHMs and HAs. Rare examples of CHMs displaying aberrant (positive) p57 expression attributable to retention of the maternal copy of chromosome 11 have been reported [17]. Conversely, PHMs with loss of maternal chromosome 11 may show negative p57 expression [18]. Several studies have evaluated p57 immunohis-

tochemical staining and have revealed high concordance of the results with morphology, ploidy, and molecular genotyping studies [19, 20]. p57 immunostaining can also be helpful when refining the diagnosis of some morphologically challenging cases and for detecting androgenetic cell lines in mosaic/chimeric conception cases [21]. The present authors identified loss of p57 expression in all CHM cases. In the present cases, nearly all p57 staining results were readily interpretable as negative or positive, with the exception of two equivocal results encountered in cases proven to be PHMs by molecular genotyping. The authors confirmed that p57 is of no value when trying to distinguish PHM from HA, although it is helpful to distinguish CHM from PHM and HA, as both conditions show similar p57 expression patterns.

CD117 is a surface marker for embryonic, hematopoietic, and mesenchymal stem cells; it allows cells to remain in their undifferentiated state [22]. The interaction between CD117 with its ligand stem cell factor (SCF) promotes phosphorylation and activation of intracytoplasmic signal cascades essential for embryogenesis, hematopoiesis, proliferation, and migration of germ cells. In addition, binding of SCF to CD117 promotes tumour growth by promoting proliferation and/or by protecting tumour cells from death. Few studies have investigated CD117 expression in HMs. Ahmed *et al.* found that the CHM trophoblast cells express CD117 with variable intensity and localisation, but no comparison was made with HAs or with PHMs [23]. A recent study found that CD117 expression pattern does not differ among CHMs, PHMs and normal pregnancy [24]. The authors found a significantly increased CD117 expression pattern in CHMs and PHMs compared with that in HAs. This finding suggests that over-expression of CD117 may play a critical role in the aggressive behavior of CHMs.

Bcl-2 is a type of proliferation or maturation-related marker of trophoblasts that shows decreased expression along with terminal differentiation and maturation [25]. Previous studies have reported contradictory results in relation to Bcl-2 expression for discriminating molar and non-molar pregnancies. A study by Fulop *et al.* demonstrated significantly stronger Bcl-2 protein expression in CHMs and choriocarcinoma compared with that in both normal placentas and PHMs [26]. Al-Bozom showed that Bcl-2 staining pattern does not differ among CHMs, PHMs, and HAs [27]. Hussein reported strong Bcl-2 expression in chorionic villi from first trimester pregnancy terminations compared with CHMs and PHMs. Author suggested that the relatively moderate Bcl-2 expression in partial and CHMs may prevent apoptotic cell death of these atypical trophoblastic cells, allowing them to acquire a more malignant potential [12]. The present results demonstrated a significant decrease in Bcl-2 expression in CHMs and PHMs compared with HAs, inferring an increased apoptotic profile in molar pregnancy. Some of these discrepancies can be attributed to differences in the immunohistochemical staining method and the evaluation of Bcl-2 expression. The present authors propose that the variations in Bcl-2 expression among CHMs, PHMs, and HAs may be used as a potential adjunctive diagnostic tool to discriminate the three entities.

Immunohistochemical analysis of c-erbB-2 overexpression in HMs has been proposed as a predictor of persistent trophoblastic disease in several studies. Some authors found increased c-erbB-2 expression in CHM that progressed to a gestational trophoblastic tumour compared with those with spontaneous remission [28, 29]; however, this finding has not been corroborated [11]. Fulop *et al.* found that c-erbB-2 staining was significantly stronger in cases of CHM and choriocarcinoma compared with normal placenta and PHM [26]. Bauer *et al.* reported that high c-erbB-2 expression in combination with DNA hyperploidy is associated with more aggressive behavior of the GTD [30]. The present study is the first attempt to assess the value of c-erbB-2 expression to distinguish HMs from HAs. The present results demonstrate a higher percentage of c-erbB-2 expression in CHMs compared with PHMs, which supports the more aggressive characteristics of CHMs. In addition, immunohistochemical staining for c-erbB-2 provided useful diagnostic information to distinguish among CHMs, PHMs, and HAs.

Previous studies have shown that histological evaluations in combination with techniques that determine DNA content (ploidy) improve the accuracy of diagnosing HMs. These ancillary molecular techniques include conventional cytogenetics (karyotyping), flow cytometry, image analysis, and FISH [31-33]. DNA ploidy analysis can readily distinguish triploid PHM from diploid conceptions but cannot distinguish between CHM and HA because both are diploid. The most recent ancillary technique, molecular genotyping using PCR amplification of STR loci, allows for determination of both ploidy and the maternal/paternal contributions of chromosome complements. Unlike karyotyping, molecular genotyping does not require fresh tissue, as it can be performed on routine formalin-fixed paraffin-embedded material, making it particularly suitable for clinical practice. Other ploidy techniques, including FISH, can be performed on fresh paraffin-embedded tissue but occasionally produce results that are difficult to interpret because of maternal tissue contamination. Recent data suggest that molecular genotyping may have advantages over other molecular methods used to distinguish androgenetic diploidy, diandric triploidy, and biparental diploidy, which are characteristic of CMs, PHMs and HAs, respectively [34,35]. One study proposed a diagnostic working algorithm in combination with molecular genotyping and p57 immunohistochemistry to refine the diagnosis of HMs [36]. However, molecular genotyping methods are technically difficult, relatively expensive, time consuming, and not universally

available. Another limitation of genotyping is that maternal decidual tissue free of fetal tissue must be present for comparison of villous and paternal alleles. In the present study, STR genotyping was performed on the equivocal cases reported herein due to mixed histological features that did not conform to the expected findings for typical CHMs, PHMs, and HAs, and its use was crucial to determine the correct diagnosis.

Conclusion

Immunohistochemical examination of p57, c-erbB-2, CD117, and Bcl-2 expression is a relatively simple, reliable, and cost-efficient procedure to definitively distinguish among CHM, PHM, and HA. However, molecular techniques are still required for evaluating some challenging cases.

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Association of estrogen receptor-beta (ESR2) polymorphism and cancer risk: a meta-analysis

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Summary

Estrogen signal medicated by estrogen receptor (ER), which is involved in various diseases related to steroid hormone, such as cancer. A number of association studies have focused on ESR2 polymorphisms to investigate the relationship with cancer risk. However, the results are inconsistent and inconclusive. To examine this controversy, 33 studies were enrolled for the pooled analysis for three polymorphisms (rs3020450, rs4986938, and rs1256049) in cancer risk using odds ratios (ORs) with 95% confidence intervals (CIs). Regarding rs4986938, A allele was associated with decreased breast cancer. Ethnicity subgroup analysis observed a decreased risk in both Asian and Caucasian descendent. Regarding rs1256049, cancer type subgroup analysis revealed a significant association with increased prostate and endometrial cancer risk. rs3020450 was not associated with cancer risk in any model. Further studies for clarifying the roles of ESR2 polymorphisms in cancer risk seem of vital importance.

Key words: ESR2; Polymorphism; Cancer risk; Meta-analysis.

Introduction

Cancer is one of the most serious medical problems threatening human life and ranks as the leading cause of death. As is well known, many factors that contribute to cancer occurrence have been reported, such as lifestyle, tobacco, alcohol addiction, environment, and so on [1]. Moreover, recent studies indicated that estrogen was associated with an increased risk of multiple types of cancer, especially breast and prostate cancer and may represent a leading preventable cause of death [2, 3]. Estrogen is mediated by the estrogen receptor (ESR), which interacts with other cell-signaling pathways to influence cell behavior. There are two major ESR subtypes: ESR1 and ESR2, which are encoded by two separate genes located on chromosome 6q25.1 and chromosome 14q23.1, respectively [4, 5]. Since ESR2 was identified in 1996 [6], there has been mounting evidence that the genetic variants in ESR2 gene have an influence on body weight [7], Alzheimer's disease, [8], anorexia nervosa [9], and so on, whereas the specific functions of ESR2 in carcinogenesis are not yet known. Currently, related studies have drawn close attention to ESR2 polymorphisms (rs3020450, rs4986938, and rs1256049) which were thought to be associated with the risk of various cancers, such as breast and prostate cancer, uterine fibroids, and other cancers; however, the results were generally inconclusive and inconsistent. The inconsistencies in previous studies might be due to small sample sizes, different research populations, and random errors. Therefore, the present authors performed a comprehensive meta-analysis to derive a more precise estimation of the correlation between these three polymorphisms and the cancer risks.

Materials and Methods

Identification and selection of eligible studies

The following bibliographic databases were searched by using the combined words ''ESR2/ERβ/ER-beta/estrogen receptor beta'', ''cancer'' or ''carcinoma'', ''genetic variation'' or ''polymorphism''. A comprehensive systematic bibliographic search was applied through the medical databases PubMed, CNKI, and WanFang for all publications up to June 2014. The criteria for acceptance of the studies were as follows: (1) studies evaluated ESR2 (rs3020450, rs4986938, and rs1256049) gene polymorphisms and available cancer risk; (2) case-control studies; (3) the numbers of the genotype or allele were reported in the article or could be obtained from authors or other source; (4) available genotype frequency. Moreover, the studies were eliminated as follows: (1) case-only studies, case reports, editorials, and review articles (including meta-analyses); (2) studies without raw data available; (3) duplicated studies.

Data extraction

Two authors (Wenkai Xia and Weidong Mao) independently extracted all the data based on the inclusion criteria listed above. All disagreements regarding eligibility were resolved by discussion with a third author (Qiwen Deng). Any study with incorrect or inconsistent data was excluded. The following variables were extracted from each study if available: first author's last name and the year of publication, country of subjects, cancer type, genotyping method and ethnicity of the population, matching numbers

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Table 1. — *Characteristics of studies included in the meta-analysis.*

Year	Cancer	Country	Ethnicity	Source of control	Genotyping method	Polymorphism sites	Cases	Controls
2009	Uterine fibroids	Germany	Caucasian	HB	PCR-ARMS	rs3020450	101	102
2010	Uterine fibroids	China	Asian	HB	TaqMan	rs4986938, rs1256049	92	193
2010	TGCT	Italy	Caucasian	HB	TaqMan	rs1256049	234	218
2009	PC	France	Caucasian	HB	Taqman	rs4986938, rs1256049	382	381
2010	PC	Japanese	Asian	HB	Taqman	rs1256049	180	177
2007	PC	[1]Mix	[2]Mix	HB	Taqman	rs3020450, rs4986938, rs1256049	8323	9412
2009	PC	USA	Caucasian	PB	TaqMan	rs4986938	219	370
2012	PC	Iran	Asian	PB	PCR-RFLP	rs4986938,rs1256049	162	324
2005	PC	China	Asian	HB	TaqMan	rs1256049	40	86
2004	PC	Japan	Asian	HB	TaqMan	rs1256049	136	236
2014	OC	Germany	Caucasian	HB	PCR-ARMS	rs3020450	184	182
2009	OC	USA	Mix	PB	TaqMan	rs3020450	147	251
2009	OC	USA	Caucasian	PB	TaqMan	rs3020450	72	146
2009	OC	USA	Asian	PB	TaqMan	rs3020450	94	172
2010	LC	USA	Caucasian	PB	Taqman	rs3020450, rs4986938, rs1256049	1021	826
2011	Melanoma	Italy	Caucasian	HB	TaqMan	rs4986938	112	195
2012	LC	Singapore	Asian	PB	TaqMan	rs4986938, rs1256049	702	1578
2009	HCC	China	Asian	HB	TaqMan	rs4986938, rs1256049	100	100
2012	GBC	India	Asian	HB	PCR-LDR	rs1256049	410	220
2009	EC	Australia	Caucasian	HB	TaqMan	rs4986938, rs1256049	191	291
2004	EC	USA	Caucasian	PB	Taqman	rs1256049	220	661
2013	EC	Germany	Caucasian	HB	PCR-ARMS	rs3020450	135	135
2013	EC	China	Asian	HB	TaqMan	rs4986938, rs1256049	60	60
2011	CRC	Germany	Caucasian	HB	PCR-ARMS	rs4986938	676	669
2010	BTC	China	Asian	PB	TaqMan	rs4986938, rs1256049	411	786
2009	BC	Germany	Caucasian	HB	PCR-ARMS	rs3020450	318	318
2006	BC	USA	Caucasian	PB	TaqMan	rs4986939	88	1272
2007	BC	[1]Mix	Caucasian	PB	Taqman	rs3020450, rs4986938, rs1256049	5789	7761
2005	BC	Sweden	Caucasian	HB	PCR-RFLP	rs4986938, rs1256049	723	480
2003	BC	China	Asian	PB	PCR-RFLP	rs1256049	1113	1209
2009	BC	Sweden	Caucasian	PB	Sequencing	rs3020450	538	1073
2009	BC	Japan	Asian	PB	PCR-LDR	rs4986938, rs1256049	388	388
2009	BC	Japan	Mix	PB	PCR-LDR	rs4986938, rs1256049	458	458
2003	BC	Sweden	Caucasian	HB	PCR-RFLP	rs4986938, rs1256049	219	238
2009	BC	India	Asian	HB	PCR-RFLP	rs4986938	248	249
2009	BC	Gernany	Caucasian	PB	TaqMan	rs4986938, rs1256049	3919	7421

[1] Mixed United States and Europe, [2] Mixed population including Caucasian, Asian, and African.
TGCT: testicular germ cell tumor; OC: ovarian cancer; BTC: biliary tract cancer; BC: breast cancer; CRC: colorectal cancer; EC: endometrial cancer; HCC: hepatocellular cancer; PC: prostate cancer; LC: lung cancer; GBC: gallbladder carcinoma; PB: population based; HB: hospital based; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR: polymerase chain reaction-ligation detection reaction; PCR-ARMS: polymerase chain reaction-amplification refractory mutation system.

of genotyped cases and controls, and polymorphism site (Table 1). If difference and discrepancies were existed after data collection, discussion was carried out to reach a consensus.

Statistical analysis

Odds ratio (OR) with its 95% confidence intervals (CI) was calculated to assess the overall association of ESR2 rs3020450, rs4986938, and rs1256049 polymorphisms with cancer risk. The pooled ORs were calculated for the risks of carriage of the mutant allele on cancers compared with the wide-type homozygote, followed by evaluating the risk in the recessive model and dominant model. Stratified analysis was also performed according to cancer type (endometrial, prostate, breast, and other cancer groups which combined the cancer types containing less than two individual studies), source of control and genotyping method. Chi-square test based Q-statistic test was used to evaluate heterogeneity across the studies [10], and was considered significant if $p_{\text{heterogeneity}} < 0.05$. Both fixed-effects (the Mantel-Haenszel method) and random effects (the DerSimonian and Laird method) models were used to pool the results [10]. A fixed-effect model was employed when no heterogeneity existed. Otherwise, the random-effect model was employed to pool the results. Publication bias was applied by funnel plots and the Egger's linear regression test. For the controls of each study, the genotype frequencies of the three polymorphisms of ESR2 were assessed for Hardy-Weinberg equilibrium using a web-based program. All statistical tests were performed with STATA version 11.0.

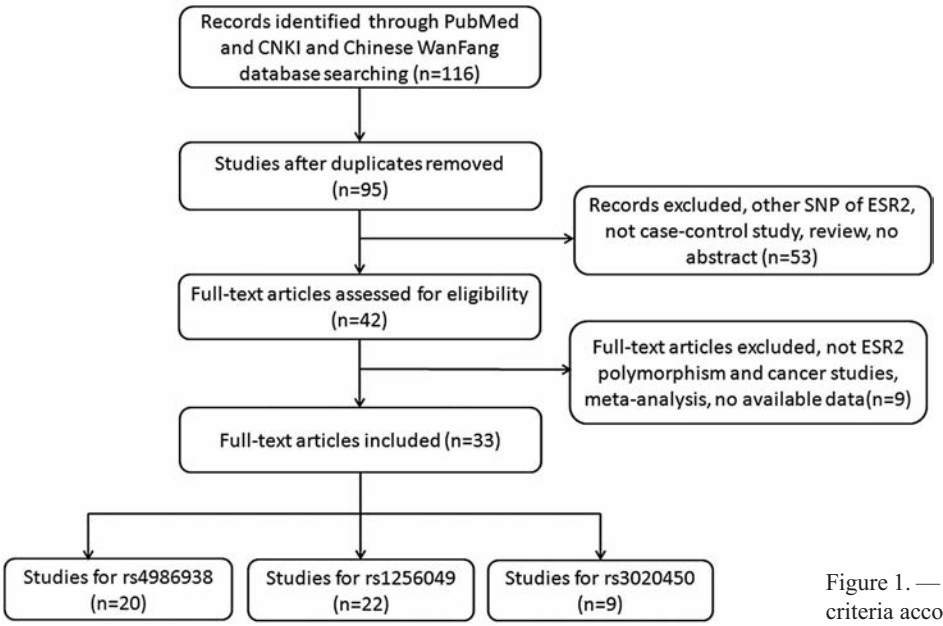

Figure 1. — Flow chart of studies identified with criteria according to inclusion and exclusion.

Results

Characteristics of studies

This study enrolled 33 eligible papers [2, 12-41] (Figure 1) according to the inclusion criteria. For ESR2 rs3020450 polymorphism, nine studies including 14,369 cases and 17,661 controls were classified into ovarian cancer (two studies), breast cancer (three studies), and the others, which were categorized into "other cancers". Meanwhile, there were nine studies of Caucasian descendent, two mixed descendent, and one Asian descendent. For ESR2 rs4986938 polymorphism, 20 studies provided available data, 22,833 cases and 30,319 controls included which were classified into prostate cancer (four studies), lung cancer (two studies), endometrial cancer (two studies), breast cancer (seven studies), and others (five studies) which were categorized into "other cancers". Meanwhile, these studies with data of studies of 12 Caucasian descendent, eight of Asian descendent, and two mixed descendent were collected for the pooled analysis. For ESR2 rs1256049 polymorphism, 22 studies including 22,722 cases and 28,952 controls consisted of Caucasian descendent (12 studies), Asian descendent (13 studies), and mixed descendent provided available data, which related to prostate cancer (six studies), lung cancer (two studies), breast cancer (six studies), and other cancers. Furthermore, the controls of most studies were population-based and the main genotyping method was PCR-RFLP (Table 1).

Main results

For ESR2 rs4986938 polymorphism, subgroup analysis revealed a low decreased risk for breast cancer in heterozygote comparison (AG *vs.* GG: OR = 0.94, 95% CI: 0.90-1.0, $p_{heterogeneity} = 0.62$) and dominant model comparison (AA + AG *vs.* GG: OR = 0.94, 95% CI: 0.90−0.99, $p_{heterogeneity} = 0.285$) (Table 2 and Figure 2). In a stratified analysis by ethnicity, a decreased risk was observed for Asian descendent (AA *vs.* GG: OR = 0.56, 95% CI: 0.39−0.82, $p_{heterogeneity} = 0.096$; AA *vs.* AG + GG: OR = 0.76, 95% CI: 0.63−0.92, $p_{heterogeneity} = 0.065$). Moreover, a decreased risk was observed for Caucasian descendent (AA + AG *vs.* GG: OR = 0.96, 95% CI: 0.92−1.00, $p_{heterogeneity} = 0.562$). In addition, cancer type subgroup analysis revealed A allele was associated with decreased breast cancer (OR = 0.96, 95% CI: 0.93−1.00, $p_{heterogeneity} = 0.088$).

For ESR2 rs1256049 polymorphism, cancer type's subgroup analysis revealed a significant association in the comparison of homozygote model (AA *vs.* GG: OR = 3.5, 95% CI: 1.27-9.64, $p_{heterogeneity} = 0.842$), heterozygote model (AG *vs.* GG: OR = 1.53, 95% CI: 1.03−2.25, $p_{heterogeneity} = 0.305$), and dominant model (AA + AG vs GG: OR = 1.60, 95% CI: 1.09−2.35, $p_{heterogeneity} = 0.205$) in endometrial cancer. Similarly, an increased risk was observed for the comparison of homozygote model (AA *vs.* GG: OR = 1.40, 95% CI: 1.16−4.49, $p_{heterogeneity} = 0.411$) with recessive model (AA *vs.* AG + GG: OR = 1.50, 95% CI: 1.10−2.04, $p_{heterogeneity} = 0.654$) in prostate cancer (Table 3 and Figure 3). In a stratified analysis by ethnicity, there was no association between ESR2 rs1256049 and cancer risk.

For overall analysis, results of pooled analysis revealed no significant associations between the genotypes of ESR2 rs3020450 polymorphism and cancer risk in all genetic models (shown in Table 4).

Table 2. — *Stratified analyses of ESR2 rs4986938 polymorphism and cancer risk.*

Variables	Cases/controls	A/A *vs.* G/G		A/G *vs.* G/G		A/A *vs.* (G/G+G/A)		(A/A+G/A) *vs.* G/G	
		OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]
Total	22833 / 30319	0.96 (0.9-1.01)	0.109	0.98 (0.94-1.02)	0.178	0.97 (0.92-1.02)	0.245	0.98 (0.94-1.01)	0.07
Cancer type									
LC	1565 / 1790	0.96 (0.73-1.26)	0.476	1.04 (0.88-1.23)	0.357	0.97 (0.76-1.24)	0.446	1.03 (0.88-1.20)	0.427
PC	8801 / 10233	0.96 (0.74-1.23)[c]	0.026	1.03 (0.97-1.1)	0.305	0.96 (0.75-1.24)[c]	0.019	1.03 (0.97-1.09)	0.323
BC	10837 / 16021	0.94 (0.87-1.02)	0.161	**0.94 (0.90-1.00)**[b]	0.62	0.96 (0.89-1.03)	0.271	**0.94 (0.90-0.99)**[b]	0.285
EC	248 / 346	0.79 (0.46-1.36)	0.444	0.83 (0.57-1.22)	0.639	0.91 (0.58-1.43)	0.444	0.82 (0.57-1.17)	0.506
other	1382 / 1929	0.96 (0.74-1.25)	0.248	0.92 (0.76-1.11)	0.054	0.98 (0.82-1.18)	0.545	0.9 (0.61-1.33)	0.025[c]
Ethnicity									
Asian	1996 / 3050	**0.56 (0.39-0.82)**[b]	0.096	1.01 (0.86-1.19)	0.079	**0.76 (0.63-0.92)**[b]	0.065	0.93 (0.70-1.22)[c]	0.031
Caucasian	18331 / 24521	0.96 (0.90-1.02)	0.752	0.96 (0.92-1.01)	0.616	0.98 (0.92-1.03)	0.931	**0.96 (0.92-1.00)**[b]	0.562
Mixed	2506 / 2748	1.16 (0.93-1.45)	0.471	1.10 (0.98-1.24)	0.917	1.12 (0.90-1.39)	0.459	1.11(0.99-1.24)	0.755

LC: lung cancer, PC: prostate cancer, BC: breast cancer, EC: endometrial cancer. [a] *p* value of Q test for heterogeneity test; [b] Statistically significant results; [c] Random-effect model was applied when *p* value for heterogeneity < 0.05, otherwise, fixed-effect model was applied.

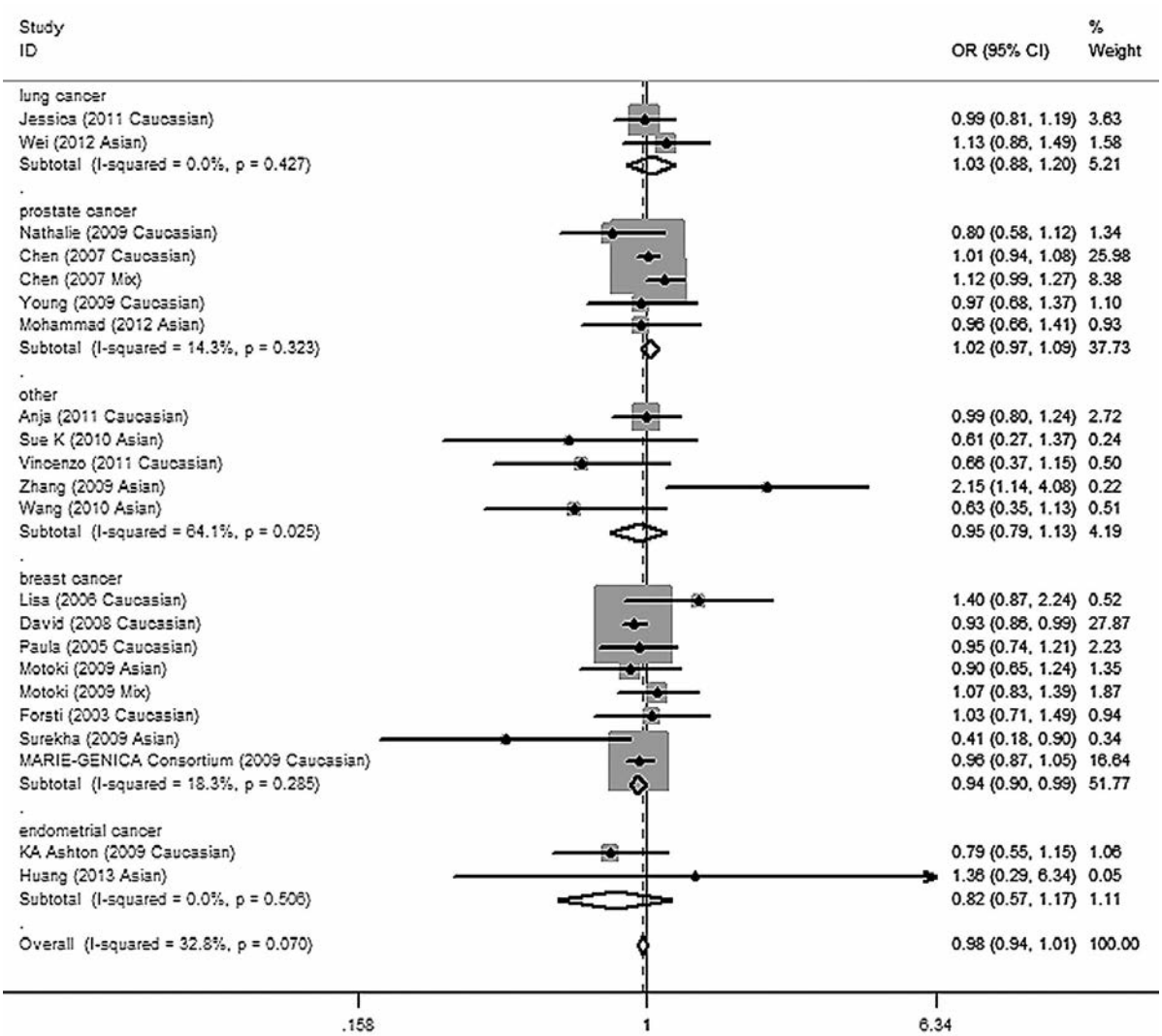

Figure 2. — Forest plots of effect estimates for cases and controls of 22 individual studies for rs4986938 stratified by cancer type (AA + GA *vs.* GG). For each study, the estimate of OR and its CI is plotted with a box and a horizontal line. Filled diamond pooled OR and its 95% CI.

Table 3. — *Stratified analyses of the ESR2 rs1256049 polymorphism and cancer risk.*

Variables	Cases/controls	A/A *vs.* G/G		A/G *vs.* G/G		A/A *vs.* (G/G+G/A)		(A/A+G/A) *vs.* G/G	
		OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]
Total	22673 / 28909	1.07 (0.75-1.54)[c]	0	0.93 (0.82-1.06)[c]	0	1.12 (0.84-1.48)[c]	0	0.94 (0.82-1.08)[c]	0
Cancer type									
lung cancer	1563 / 1779	1.13 (0.82-1.56)	–	1.01 (0.80-1.27)	–	1.13 (0.84-1.51)	–	1.01 (0.84-1.23)	0.606
prostate cancer	7796 / 8927	**1.40 (1.02-1.91)**[b]	0.411	0.91 (0.73-1.14)[c]	0.011	**1.50 (1.10-2.04)**[b]	0.654	0.98 (0.82-1.18)[c]	0.048
breast cancer	11652 / 15726	0.47 (0.19-1.13)[c]	0	0.91 (0.73-1.14)[c]	0	0.57 (0.26-1.23)[c]	0	0.83 (0.62-1.12)[c]	0
endometrial cancer	471 / 1010	**3.50 (1.27-9.64)**[b]	0.842	**1.53 (1.03-2.25)**[b]	0.305	1.72 (0.85-3.68)	0.549	**1.60 (1.09-2.35)**[b]	0.205
other	1240 / 1510	0.84 (0.27-2.60)[c]	0.001	0.72 (0.48-1.09)[c]	0.045	0.95 (0.42-2.16)[c]	0.01	0.72 (0.45-1.15)[c]	0.01
Ethnicity									
Asian	4085 / 5191	1.15 (0.88-1.51)[c]	0.008	0.89 (0.76-1.04)[c]	0.042	1.09 (0.96-1.24)	0.089	0.94 (0.81-1.09)[c]	0.022
Caucasian	17401 / 22337	0.23 (0.02-2.39)[c]	0	0.93 (0.72-1.19)[c]	0	0.34 (0.04-2.93)[c]	0	0.86 (0.65-1.15)[c]	0
Mixed	458 / 458	1.10 (0.72-1.69)	–	1.07 (0.81-1.40)	–	1.87 (0.53-6.65)	–	1.03 (0.79-1.33)	–
Africa	778 / 966	1.87 (0.53-6.65)	–	1.00 (0.77-1.31)	–	1.07 (0.71-1.61)	–	1.07 (0.83-1.08)	–

LC: lung cancer, PC: prostate cancer, BC: breast cancer, EC: endometrial cancer; [a] *p* value of Q test for heterogeneity test; [b] Statistically significant results; [c] Random-effect model was applied when *p* value for heterogeneity < 0.05; otherwise, fixed-effect model was applied.

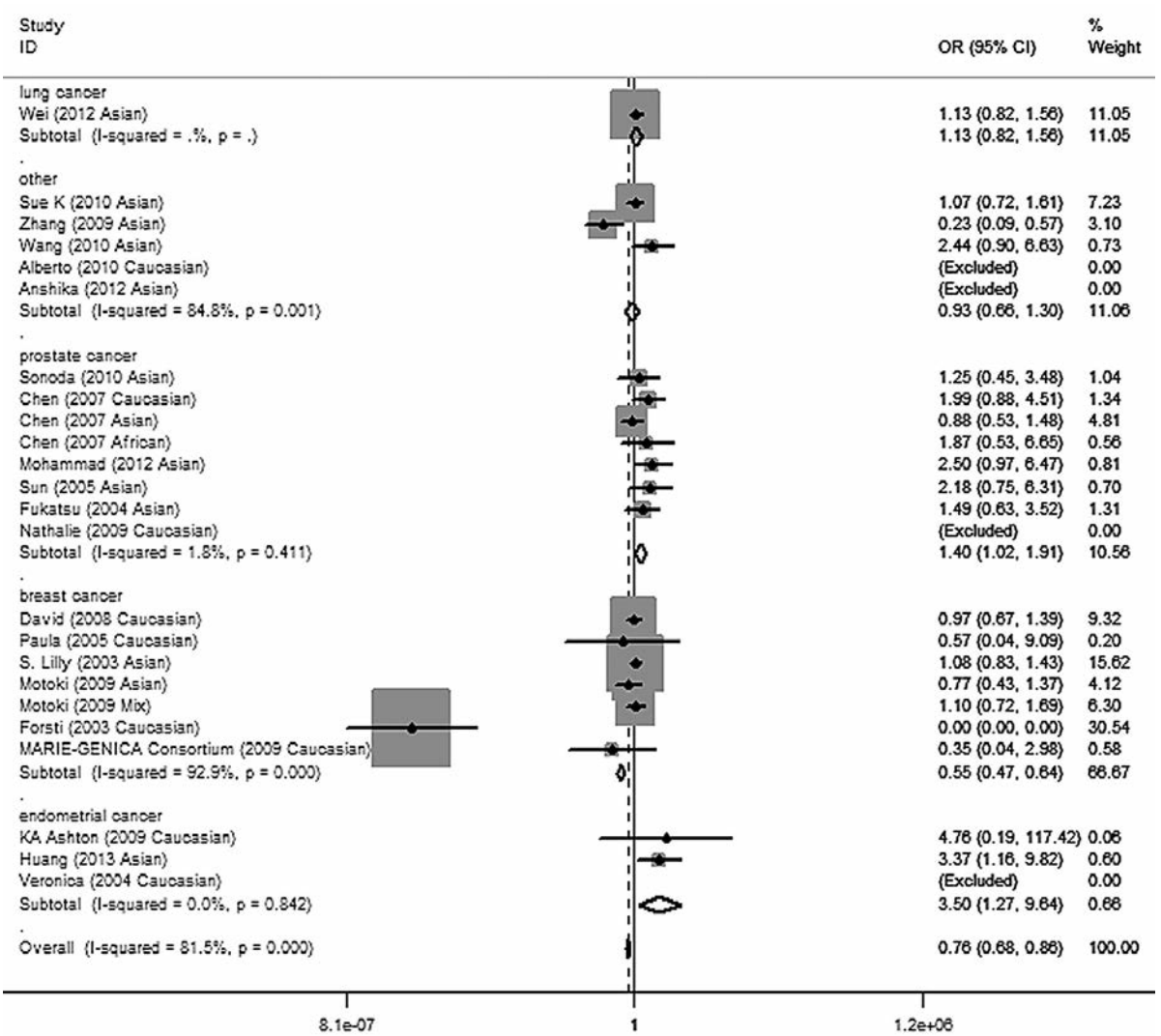

Figure 3. Forest plots of effect estimates for cases and controls of 25 individual studies for rs4986938 stratified by cancer type (AA *vs.* GG). For each study, the estimate of OR and its CI is plotted with a box and a horizontal line. Filled diamond pooled OR and its 95% CI.

Table 4. — *Stratified analyses of the ESR2 rs3020450 polymorphism and cancer risk.*

Variables	Cases/controls	A/A *vs.* G/G		A/G *vs.* G/G		A/A *vs.* (G/G+G/A)		(A/A+G/A) *vs.* G/G	
		OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]	OR (95% CI)	*p* [a]
Total	16417 / 19956	0.98 (0.92-1.06)	0.557	1.01 (0.97-1.05)	0.57	0.98 (0.91-1.05)	0.465	1.00 (0.96-1.05)	0.696
Cancer type									
OC	497 / 751	1.12 (0.72-1.73)	0.148	0.89 (0.59-1.36)[c]	0.046	1.07 (0.70-1.63)	0.102	0.94 (0.74-1.19)	0.085
BC	6481 / 8918	0.94 (0.84-1.05)	0.491	1.00 (0.94-1.07)	0.962	0.94 (0.84-1.04)	0.507	0.99 (0.93-1.05)	0.824
PC	8182 / 9224	1.00 (0.90-1.11)	0.818	1.03 (0.97-1.10)	0.612	0.99 (0.90-1.09)	0.929	1.02 (0.96-1.09)	0.614
other cancer	1257 / 1063	1.11 (0.84-1.47)	0.654	0.96 (0.81-1.15)	0.975	1.13 (0.87-1.47)	0.58	0.99 (0.84-1.17)	0.995
Ethnicity									
Asian	94 / 172	2.78 (0.87-8.86)	–	0.66 (0.38-1.22)	–	3.11 (0.99-9.79)	–	0.85 (0.50-1.47)	–
Caucasian	14128 / 17238	0.98 (0.91-1.06)	0.835	1.01 (0.96-1.06)	0.8	0.97 (0.91-1.05)	0.749	1.00 (0.96-1.05)	0.892
Mixed	2195 / 2546	0.99 (0.81-1.21)	0.126	1.03 (0.91-1.12)	0.077	0.97 (0.80-1.18)	0.186	0.87 (0.55-1.37)[c]	0.04

OC: ovarian cancer, PC: prostate cancer, BC: breast cancer; [a] *p* value of Q test for heterogeneity test; [b] Statistically significant results;
[c] Random-effect model was applied when *p* value for heterogeneity < 0.05, otherwise, fixed-effect model was applied.

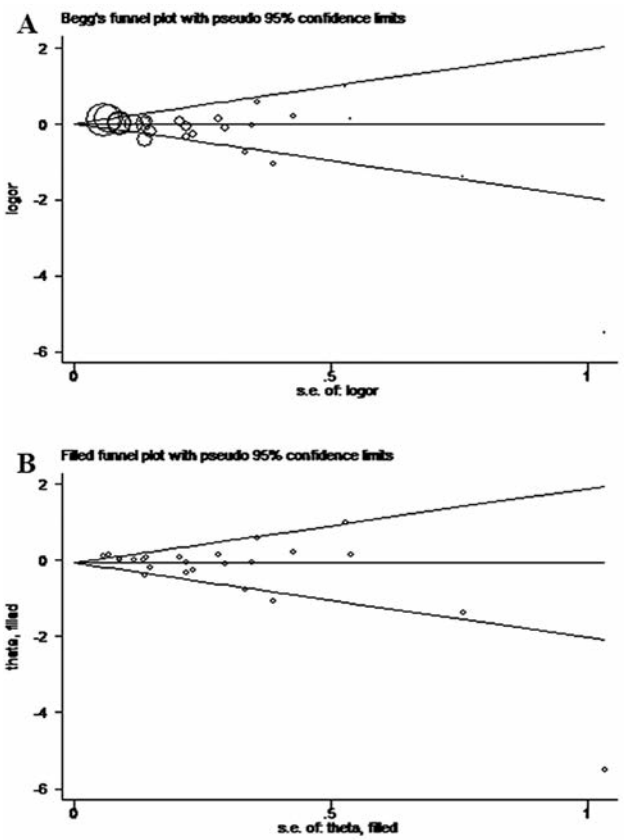

Figure 4. — Begg's funnel plot of Egger's test for publication bias tests for heterozygote comparison in ESR2 rs1256049. Each *circle* represents as an independent study for the indicated association. Log [OR], natural logarithm of OR. *Horizontal lines* mean effect size. A: Begg's funnel plot of publication bias test. B: Begg's funnel plot of publication bias test after trim-and-fill method.

Test of heterogeneity

For overall studies of ESR2 rs1256049 polymorphism, a significant heterogeneity was apparent among homozygous comparison (AA vs GG: $p_{\text{heterogeneity}}$ =0.000), heterozygote comparison (AG *vs.* GG: $p_{\text{heterogeneity}}$ =0.000), recessive comparison (AA *vs.* AG + GG: $P_{\text{heterogeneity}}$ =0.000), dominant model (AA + AG *vs.* GG: $P_{\text{heterogeneity}}$ =0.000)

There was no apparent heterogeneity for overall studies of ESR2 rs4986938 and ESR2 rs3020450.

Table 5. — *Egger's test for three polymorphisms of ESR2.*

Polymorphism	Egger's test	Homozygous	Heterozygous	Recessive	Dominant
rs3020450	*t*	0.96	-0.74	1.01	-0.37
	p	0.358	0.476	0.337	0.719
rs4986938	*t*	-0.66	-0.37	-0.27	-0.044
	p	0.52	0.715	0.788	0.665
rs1256049	*t*	-0.55	-2.62	-0.23	-2.09
	p	0.59	**0.016**	0.824	0.058

Sensitivity analysis and publication bias

Sensitivity analysis was performed to assess the stability of these results and to find the source of the heterogeneity by sequential removal of individual eligible study. The results of sensitivity analysis were obtained after sequentially excluding each case-control study, indicating the stability of the results.

Begg's funnel plot and Egger's test were performed to assess the publication bias. The shape of the funnel plot indicated obvious asymmetry in ESR2 rs1256049 heterozygous model comparison and dominant model comparison (Figure 4A). Thus, Egger's test was used to provided statistical evidence of funnel plot asymmetry (t = -2.62, p = 0.016) (shown in Table 5), which suggested the existence of publication bias in the meta-analysis. To adjust this bias, a trim-and-fill method mentioned by Duval and Tweedie [42] was utilized (Figure 4B). As a result, the conclusion with or without the trim-and-fill method did not change, indicating that the present results were statistically robust. While the shapes of the funnel plots did not reveal any evidence of obvious asym-

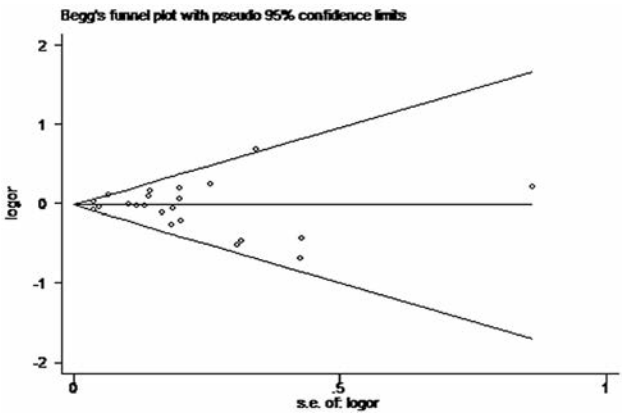

Figure 5. — Begg's funnel plot of the Egger's test of allele comparison for publication bias for AG versus GG in ESR2 rs4986938 polymorphism.

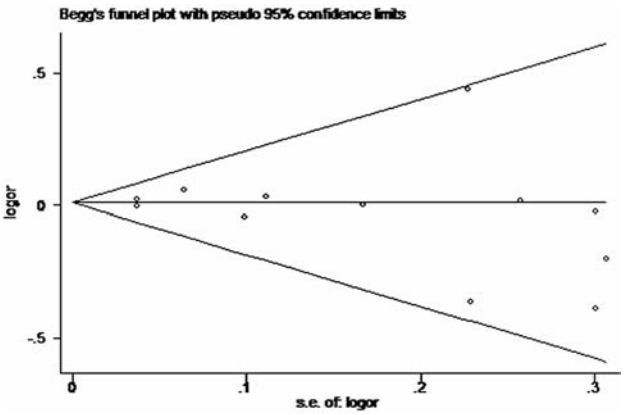

Figure 6. — Begg's funnel plot of the Egger's test of allele comparison for publication bias for AG versus GG in ESR2 rs3020450 polymorphism.

metry in all genetic models of ESR2 rs3020450 and ESR2 rs4986938 polymorphisms (Figures 5, 6). In addition, all models of ESR2 rs3020450 and ESR2 rs4986938 did not show any evidence of publication bias ($p > 0.05$) (Table 5).

Discussion

Thirty-three studies were identified according to the acceptance and exclusion criteria to investigate the relationship between the genetic variants in the ESR2 gene and cancer risk. There was a correlation between estrogens and cancer risks. Estrogen metabolism was related to vitamin D, insulin sensitivity, and fat metabolism as well as inflammation development which closely linked with cancer occurrence [43]. Estrogens have significant direct and/or indirect effects on development and progression of cancer, in which ESR2 was a key factor [44, 45]. To date, it is known that the genetic polymorphisms in ESR2 gene locate on chromosome14 and can change the stability of the transcript [5, 26, 46]. It was not difficult to observe that this evidence supported the present results regarding the association between ESR2 rs4986938, rs1256049 and rs3020450, and cancer occurrence.

As for the ESR2 rs4986938 polymorphism, subgroup study revealed that there was only a single comparison model (GA + AA *vs.* GG) in Caucasian descendent showed the significant association with cancer risk. Meanwhile, significant associations were found in Asian descendent for the comparison of AA *vs*. GG and AA *vs.* GA + GG, which suggested ethnic differences did not influence the cancer risk. Significant results of different genetic models, however, were observed in two descendent, which suggested that relatively limited study number and small sample size contributed to the results. Cancer type subgroup analysis revealed that ESR2 rs4986938 polymorphism was a protective factor in breast cancer. Recently, several studies have revealed that ESR2 rs4986938 polymorphism was associated with cancer risk [16, 26, 28, 31]. However, some studies did not demonstrate a significant association between rs4986938 and cancer risk [2, 13, 27, 29]. Inconsistent results might be caused by phytoestrogen intake and BMI in different descendent which might be critical for genetic effect. In addition, the approach to select participants and study design should also be taken into account.

As for the ESR2 rs1256049 polymorphism, cancer type subgroup analysis revealed that there existed a correlation between ESR2 rs1256049 polymorphism and the risk of prostate cancer under homozygous (AA *vs.* GG) model, it showed the same pattern of results as that under recessive model (AA *vs.* AG + GG). Meanwhile, a significant association was also observed between ESR2 rs1256049 and endometrial cancer. In the subgroup analysis by ethnicity, no significant association was associated with cancer risk in any genetic model. However, the results might be caused by relatively limited study number, only two studies of rs1256049 in subgroup analysis specific to Caucasians [2, 27]. For the ESR2 rs1256049 polymorphism, due to many conflicted results [20, 27, 35, 38], further well-designed, unbiased, large case-control studies need to be performed to confirm these results.

As for the ESR2 rs3020450 polymorphism, no significant associations were found in all comparisons. In similarly, three studies did not show a significant association by comparison ESR2 rs3020450 with ovarian cancer. In addition, no significant association was found among uterine fibroids, prostate, lung, and breast cancer [2, 15, 18, 37]. Therefore, more related studies need to further clarify the relationship between ESR2 rs3020450 polymorphism and cancer risk.

Some potential limitations of this meta-analysis should be acknowledged. First, all the eligible studies the authors searched were from the database in English and Chinese, articles with potentially high-quality data that were pub-

lished in other languages were not included in this paper because of potential medical translation inaccuracies. Second, though most controls were selected from healthy populations, there was no uniform definition of controls. Finally, some potentially suspected factors such as age, sex, living habits, menstrual history, and environmental factors were not considered so that the authors' unadjusted estimates still need further validation. However, the present meta-analysis had some advantages. First, in order to increase the statistical power of the meta-analysis significantly, the authors extracted data from as many different studies as possible. Second, all case-control studies included in this research met the authors' selection criteria well.

In conclusion, the present study demonstrated the relationship between three polymorphisms of ESR2 and cancer risk. The result indicated that rs4986938 was associated with a decreased risk of breast cancer in Caucasians and Asians, and rs1256049 polymorphism was significantly associated with prostate cancer and endometrial cancer, while rs3020450 showed no obvious associations with cancer. However, further studies based on more comprehensive and large, stratified population to facilitate evaluation the association between ESR2 and cancer risk are warranted.

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Study of clinical diagnosis of cervical glandular intraepithelial neoplasia

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Summary

Objective: To preliminarily evaluate the clinical significance of different methods in diagnosis of cervical glandular intraepithelial neoplasia (CGIN). *Materials and Methods:* Clinical manifestations, ThinPrep cytologic test (TCT), cervical biopsy, and pathological features of 106 patients with CGIN admitted to Beijing Obstetrics and Gynecology Hospital between 2008 and 2011 were retrospectively analyzed. *Results:* Among 146 cases diagnosed with CGIN, 87 (59.6%) had L-CGIN and 59 (40.4%) H-CGIN. Thirty-seven patients (25.6%) were found to have simple CGIN and 109 (74.6%) had CGIN complicated with cervical intraepithelial neoplasia (CIN). TCT revealed atypical glandular cells (AGC) in 20 patients (13.7%), six of whom had L-CGIN (6.9%) and 14 (23.7%) had H-CGIN with statistical significance between two groups ($p < 0.05$). TCT detected AGC in 13 cases (35.1%) with simple CGIN and seven with mixed CGIN (6.4%) ($P<0.05$). Endocervical curettage (ECC) revealed AGC abnormality in ten cases (25.6%). Cervical biopsy under colposcope revealed 32 patients (22.9%) had CGIN, including 15 L-CGIN (18.3%), and 17 H-CGIN (29.3%) with no statistical significance ($p > 0.05$). Among those diagnosed with CGIN by colposcope-assisted cervical biopsy, 19 (51.4%) had simple CGIN and 13 (11.9%) mixed CGIN ($p < 0.05$). *Conclusion:* Preoperative diagnostic rate of simple CGIN was higher than CGIN complicated with CIN.

Key words: Cervical glandular intraepithelial neoplasia; Endocervical glandular dysplsia; Diagnosis.

Introduction

Recently, the incidence of cervical adenocarcinoma has been increasing and the onset age is becoming younger, approximately accounting for 10%-34% of cervical cancer [1]. Cervical glandular intraepithelial neoplasia (CGIN) is precancerous lesions of adenocarcinoma and is less understood compared with cervical intraepithelial neoplasia (CIN). This study was designed to preliminarily evaluate the clinical significance of different methods in diagnosis of 146 CGIN patients admitted to the present institution from 2008 throughout 2011.

Materials and Methods

Study subject

A total of 146 patients were diagnosed with CGIN and admitted to Beijing Obstetrics and Gynecology Hospital between January 2008 and May 2011. Among them, 117 were surgically treated with cervical conization, 23 loop electrosurgical excision procedure (LEEP), and six panhysterectomy. Thirty-two patients were diagnosed with CGIN by preoperative histological examination and 114 diagnosed with CGIN postoperatively including those with CGIN complicated with CIN. Those subjects complicated with cervical squamous carcinoma and cervical adenocarcinoma were excluded from this study. All cases were aged between 22 and 67 years, 41.3 years on average.

The mean times of pregnancy were 2.5 and of delivery was 1.6. Those with menopause accounted for 15.8% and 84.2% of premenopausal women. Eighty-eight patients (60.2%) had no clinical symptoms, 25 cervical contact hemorrhage, 19 abnormal secretion, and nine abnormal vaginal bleeding. All cases with CGIN were confirmed by pathological examination.

Methods

All 146 patients underwent ThinPrep cytologic test (TCT) preoperatively. Those with AGC and undesirable transformation zone further received colposcope and cervical biopsy. Those suspected with AGC received endocervical curettage (ECC) simultaneously. Based upon histological examination, cervical conization was subsequently performed as necessary. Another ten patients with non-cervical lesions were diagnosed with CGIN by postoperative pathological examination. CGIN refers to glandular neoplasia of cervix during early stage of infiltration, divided into two levels: low-grade cervical glandular intraepithelial neoplasia (L-CGIN) and high-grade cervical glandular intraepithelial neoplasia (H-CGIN). H-CGIN included in situ adenocarcinoma.

Classification of CGIN was conducted based upon histological and cytological characteristics. Histological manifestations of H-CGIN resembled in situ adenocarcinoma. L-CGIN was characterized with certain abnormal changes. H-CGIN referred to all abnormal alterations.

Statistical analysis

SPSS 13.0 statistical software was utilized for data analysis. Chi-square and paired chi-square tests were performed. $P < 0.05$ was considered as statistical significance.

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Table 1. — *TCT outcomes.*

TCT results	Cases	AGC	non-AGC	*p* value
L-CGIN	87	6	81	< 0.05
H-CGIN	59	14	45	
Simple CGIN	37	13	24	< 0.05
Mixed CGIN	109	7	102	

Table 2. — *Cervical biopsy under colposcope in the diagnosis of CGIN of different grade*

CGIN classification	Cases	Cervical biopsy under colposcope	
		CGIN	Without CGIN
L-CGIN	82	15	67
H-CGIN	83	17	41
Total	140	32	108

Table 3. — *Relationship between preoperative and postoperative diagnosis of CGIN*

Preoperative diagnosis	Cases	Postoperative diagnosis	
		Simple CGIN	Mixed CGIN
CGIN	32	19	13
Without CGIN	114	17	96
Total	146	37	109

Results

Types of CGIN

Among 146 cases of CGIN, 37 (25.6%) had simple CGIN and 109 (74.4%) mixed CGIN, mainly complicated with CIN. Eighty-seven (59.6%) were diagnosed with L-CGIN and 59 (40.4%) with H-CGIN.

TCT

TCT revealed signs of AGC in 20 cases (13.7%), including six (6.9%) with L-CGIN and 14 (23.7%) H-CGIN; statistical significance was observed between two groups ($p < 0.05$). The detection rate of H-CGIN was significantly higher than L-CGIN by TCT. Among 37 cases diagnosed with AGC, 13 (35.1%) had simple CGIN and seven (6.4%) mixed CGIN with statistical significance ($p < 0.05$). The detection rate of TCT in simple CGIN was higher than that in mixed CGIN. The outcomes of TCT are illustrated in Table 1.

Comparison of colposcope-assisted cervical biopsy, ECC, and postoperative cervical pathological examination results

Among 146 cases, 140 underwent cervical conization. Preoperative cervical biopsy revealed 32 (22.9%) CGIN and 108 (77.1%) were diagnosed with postoperative cervical conization. These 140 patients received colposcope and cervical biopsy. Thirty-nine patients with AGC and poor transformation zone were treated with ECC. ECC revealed gland cell abnormality in ten cases (25.6%). Thirty-two patients (22.9%) were diagnosed with cervical biopsy including 15 L-CGIN (18.3%) and 17 H-CGIN (29.3%) ($\chi^2 = 2.339$, $p > 0.05$) Cervical biopsy revealed 19 cases (51.4%) had simple CGIN and 13 mixed CGIN (11.9%) ($\chi^2 = 26.259$, $p < 0.05$), as shown in Tables 2 and 3.

Discussion

CGIN and epidemiological characteristics

As the precancerous lesions of cervical adenocarcinoma, the naming and category of CGIN remain debated. In the USA and other regions, the classification criteria proposed by International Society of Gynecological Pathologists were adopted, that is, endocervical glandular dysplasia (EGD) and in situ adenocarcinoma. In the U.K. and European nations, it is named as cervical glandular intraepithelial neoplasia (CGIN), which is divided into two categories: low-grade CGIN (L-CGIN) and high-grade CGIN (H-CGIN). H-CGIN includes in situ adenocarcinoma [2]. Some scholars classified CGIN into CGIN I, II, and III. However, it is challenging to implement this standard and the diagnostic reproducibility is low in clinical practice [3]. The European classification criteria of CGIN were adopted in this study. CGIN is less commonly seen than CIN.

The ratio of cervical adenocarcinoma and squamous carcinoma is 1:5, whereas the ratio of precancerous lesions of these two cancers was 1:80 [4]. The ratio of in situ cancer and infiltrated cancer was 1:3 for adenocarcinoma and 5.25 : 1 for squamous carcinoma, and 62 (58.5%) were diagnosed with L-CGIN and 44 (41.5%) with H-CGIN. The age at onset of CGIN was 39-40 years, 39.89 years on average [5]. The mean age in this study was 41.3 years, basically consistent with previous findings. It is difficult to diagnose CGIN during early stage. Previous studies demonstrated that HPV infection is closely correlated with cervical gland lesions. HPV 16, 18, and 31 can be detected in over 80% of patients diagnosed with cervical adenocarcinoma and squamous carcinoma. However, the underlying cause remains elusive. Previous studies indicated the time of L-CGIN progressing into H-CGIN was 1.5 to three years [6]. It has been reported that HPV16 is associated with cervical in situ adenocarcinoma, and HPV18 is associated with advanced cervical adenocarcinoma [7]. In this study, 87 cases (59.6%) were diagnosed with L-CGIN and 59 (41.5%) H-CGIN. A majority of cases (60.2%) had no symptoms. Others presented with clinical symptoms mainly including vaginal secretion abnormality and contact hemorrhage.

Diagnosis of CGIN

It is a challenging task to diagnose CGIN preoperatively. A majority of CGIN is diagnosed after cervical biopsy, cervical conization or uterus excision. Previous studies indicated that approximately 46% to 72% of CGIN patients were diagnosed after excision of CIN lesions [4]. Zhang *et al.* reported that 66.7% of CGIN cases were confirmed after

treatment of CIN or benign pathological changes. In this clinical trial, the percentage of postoperative diagnosis of CGIN was 80.3%, which is consistent with previous findings. It is difficult to diagnose CGIN preoperatively due to the following reasons: 1) CGIN lesions were mainly distributed around the cervix and affected the superficial mucosa, recess gland, deep gland, CIN, SCCA, and adenocarcinoma margins. It is likely to miss diagnosis due to difficult sampling. 2) CGIN cells, endocervical cells, and endometrial cells resembled in appearance. It was difficult to differentiate from cervical squamous epithelial lesions, possibly leading to misdiagnosis. 3) CGIN is constantly complicated with CIN. Previous studies reported that up to 90% of non-infiltrated pathological changes were complicated with squamous epithelial CIN [8]. The proportion of CGIN complicated with CIN was 74.4% in this study. Severe CIN lesions may conceal the pathological changes of CGIN, mainly characterized with CIN lesions. Therefore, it is likely to miss the diagnosis of CGIN.

Diagnostic levels of pathologists

The Bethesda system (TBS) is widely applied in clinical screening of cervical pathological changes and significantly enhances the early diagnosis of cervical squamous epithelial lesions. However, the positive rate of TBS in screening of cervical lesions is low and the false-negative rate is high, which is likely to cause misdiagnosis. In addition, the detection rate of cervical AGC is equally low ranging from 0.05% to 2.1% [9]. It has been reported that the sensitivity of TCT ranged from 32.7% to 48.1%, and the specificity was 69.4%-94.4% [10]. Zhang *et al.* demonstrated the sensitivity of TCT was 33.3% in Chinese population [11]. In this study, merely 13.7% of patients presented with preoperative TCT abnormality, probably due to the majority of CGIN cases complicated with CIN, which affected the accuracy of TCT. The detection rate of L-CGIN by TCT was 6.9% (6/87) and 23.7% (14/59) for H-CGIN cases with statistical significance, suggesting that the positive rate of TCT in screening of H-CGIN is higher than L-CGIN. TCT remains the only screening approach of CGIN, whereas the sensitivity is relatively low. However, colposcope-assisted cervical biopsy and ECC have their own limitations in diagnosis of CGIN.

It is difficult to collect sampling of the lesions within the cervix under colposcope. The subjective judgement of the physicians is also likely to cause miss diagnosis. Additionally, use of ECC fails to collect the samples and the possibility of extracervical lesions could not be excluded. In this study, 39 patients received ECC, and only ten cases (25.6%) were found to have AGC, hinting a low preoperative diagnostic rate. Previous studies demonstrated that the positive predictive value of colposcope in diagnosis of squamous and glandular epithelial lesions was 93.5%, whereas 9.8% for the diagnosis of glandular epithelial lesions [12]. Other studies reported that the detection rate of glandular abnormality ranged from 35% to 70% in CGIN patients by colposcope-assisted cervical biopsy [9]. In this study, colposcope-assisted biopsy revealed glandular epithelial abnormality in 22.8% of patients and the sensitivity was not high. No statistical significance was observed in diagnostic rate of varying degree of CGIN. TCT revealed that those with AGC should undergo colposcope and cervical biopsy under direct vision to avoid the miss diagnosis. Cervical conization is of vital value in the diagnosis of CGIN.

CGIN lesions were distributed in a central and diffusive pattern, approximately 10% of CGIN lesions were located above the cervix [13]. Compared with CIN, it is more complex and difficult to diagnose CGIN. TCT and colposcope examination lack reliability. Cervical conization rather than cervical biopsy should be performed in diagnosing patients suspected with cervical glandular diseases. Kietpeerakool *et al.* [14] reported that among 51 patients diagnosed with cervical in situ adenocarcinoma by cervical conization, 22 presented with cervical glandular abnormality, 29 squamous epithelial abnormality, and 9 AGC by colposcope-assisted biopsy and/or ECC. Thirty-one patients (60.8%) were suspected with glandular diseases before surgery. Previous studies demonstrated that pap smear, colposcope-assisted biopsy, and ECC were not suitable for diagnosing cervical glandular diseases, whereas cervical conization should be considered. In this study, 140 patients underwent cervical conization including 32 (22.9%) diagnosed with glandular lesions preoperatively and 108 confirmed by pathological diagnosis following cervical conization. Therefore, cervical conization plays a pivotal role in the diagnosis of CGIN.

Preoperative diagnostic rate of simple CGIN higher than mixed CGIN

Majority of CGIN patients were complicated with CIN lesions and the percentage of simple CGIN was relatively low. CGIN patients complicated with CIN revealed that they were characterized with squamous epithelial lesions prior to examination whereas glandular epithelial pathological changes were neglected. Thus, preoperative detection rate of mixed CGIN was lower than simple CGIN. Ovanin-Rakic *et al.* [15] demonstrated that among 123 CGIN cases, 13 had adenocarcinomas in situ of the cervix (AIS), 18 glandular intraepithelial lesions (GIL) I and II, 58 AIS complicated with squamous epithelial lesions, and 34 AIS complicated with GIL I and II. The detection rate of simple AIS, GIL I, and II was 61.5% and 22%, significantly higher compared with 25.9% and 20.6% for mixed cases. Kietpeerakool *et al.* [14] reported that 20 (70.4%) among 51 cases with AIS were suspected with simple glandular lesions preoperatively, whereas 12 AIS patients (50%) were complicated with CIN. Preoperative detection rate of simple AIS was higher than that of mixed AIS. In this study, TCT revealed the signs of AGC in 37 patients (35.1%) with simple CGIN, and seven cases (6.4%) with mixed CGIN with statistical significance ($p < 0.05$). Thus, the detection

rate of AGC by TCT in simple CGIN was significantly higher than mixed CGIN. Thirty-two cases were diagnosed with CGIN by colposcope-assisted cervical biopsy including 19 (51.4%) simple CGIN and 13 (11.9%) mixed CGIN with statistical significance ($p < 0.05$), suggesting that it is much easier to identify simple CGIN than mixed CGIN. Taken together, it is difficult to preoperatively diagnose CGIN. TCT remains the only screening approach of CGIN with relatively low sensitivity. Colposcope, cervical biopsy/ECC present with a low detection rate of CGIN. For CGIN patients complicated with CIN, conventional colposcope and endocervical curettage should be performed to detect AGC. For those suspected with glandular epithelial abnormality, cervical conization should be performed to confirm the diagnosis. How to screen and diagnose CGIN during early stage and before surgery remains a challenge. Along with the improvement of cervical sampling and cell molecular diagnosis and deeper understanding of clinical and pathological physicians, preoperative detection rate of CGIN is increased, thereby reducing the incidence and mortality of infiltrated cervical adenocarcinoma.

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Case Reports

Peritoneal tuberculosis associated with adrenocorticol primitive neoplasm mimicking a peritoneal carcinosis: a case report

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Summary

The authors report a case of women, 51-year-old that presented with peritoneal tuberculosis, with concomitant adrenocortical primitive neoplasm, mistaken as peritoneal carcinomatosis, due to the failure of correct histological analysis. In fact, the critical life status, associated to increases of CA 125, typical imaging of peritoneal carcinomatosis, and presence of 75% atypical cells in ascitic fluid, induced to begin chemotherapy.

Key words: Peritoneal carcinosis; Tuberculosis; CA 125; Chemotherapy; Immunosuppression.

Introduction

Very little literature describes the capacity of peritoneal tuberculosis to mimic a peritoneal carcinomatosis [1, 2]. An elevation of CA 125 has been observed in tumoral peritoneal carcinomatosis, but has also been observed in cases of peritoneal tuberculosis. If CA 125 monitoring is well known as a means to assess response to anti-cancer therapies [3], its interest is not appreciated for the monitoring of peritoneal carcinomatosis associated with mycobacterium tuberculosis. CT-scan is not specific enough for the diagnosis of peritoneal carcinomatosis and does not allow to distinguish its origin. PET scan can be useful for diagnosis of peritoneal carcinomatosis [4] but does not prove the certitude of the diagnostic. Laparoscopy with histological analysis is the only diagnostic instrument able to provide a clear diagnosis [5].

Case Report

In July 2008, a 51-year-old women who came from Madagascar and had been living in France for about ten years, presented an important abdominal volume increase associated only with abdominal pain. The clinical history of the patient showed that a Bartholin cyst surgery had been performed in 2003, arterial hypertension controlled with drugs, and that the patient was a heavy smoker (15–20 cigarettes/day). The biological marker CA 125 was highly elevated (717 KU/L), however, no other biological anomaly was observed. Ultrasound exploration showed abundant ascites with peritoneal carcinomatosis and pleural effusion with dilatation of the mediastinum. In order to deepen the diagnosis and to expedite treatment, a CT-scan and ascitic liquid analysis were performed. The CT-scan showed a peritoneal and bilateral pleural carcinomatosis (Figure 1a), pelvic mass with uterus myomatous confirming the previous ultrasound diagnosis (Figure 1b), and a right adrenal mass of six cm, compatible with adrenocortical primitive neoplasm (Figure 1c). The biochemical, bacteriological, and cytological ascitic liquid analysis were diagnostically non-contributory, except for the presence of 75% atypical cells.

Because the clinical life status of the patient deteriorated rapidly, no coelioscopy was performed and a chemotherapy was established with carboplatin and paclitaxel (six cycles), beginning in August 2008 and ending in November 2008. During chemotherapy, a primary treatment of neutropenia (granulocyte colony stimulating factor, GCSF) was administered.

After the sixth cycle of chemotherapy, a control CT-scan showed a disappearance of abdominal ascites, important reduction of abdominal and pleural carcinomatosis (Figure 1e), and stability of size of the right adrenal mass (Figure 1f). However, bilateral pulmonary embolism was diagnosed (Figure 1d). The patient was immediately treated with anticoagulation therapy (tinzaparin sodium). A PET-scan realized in December 2008 revealed a suspected abdominal and pleural fixing, compatible with the conclusion of the CT-scan, excluding the right adrenal mass (Figure 2a). Monitoring of the biological marker CA 125 was performed prior to during and following chemotherapy (Figure 3).

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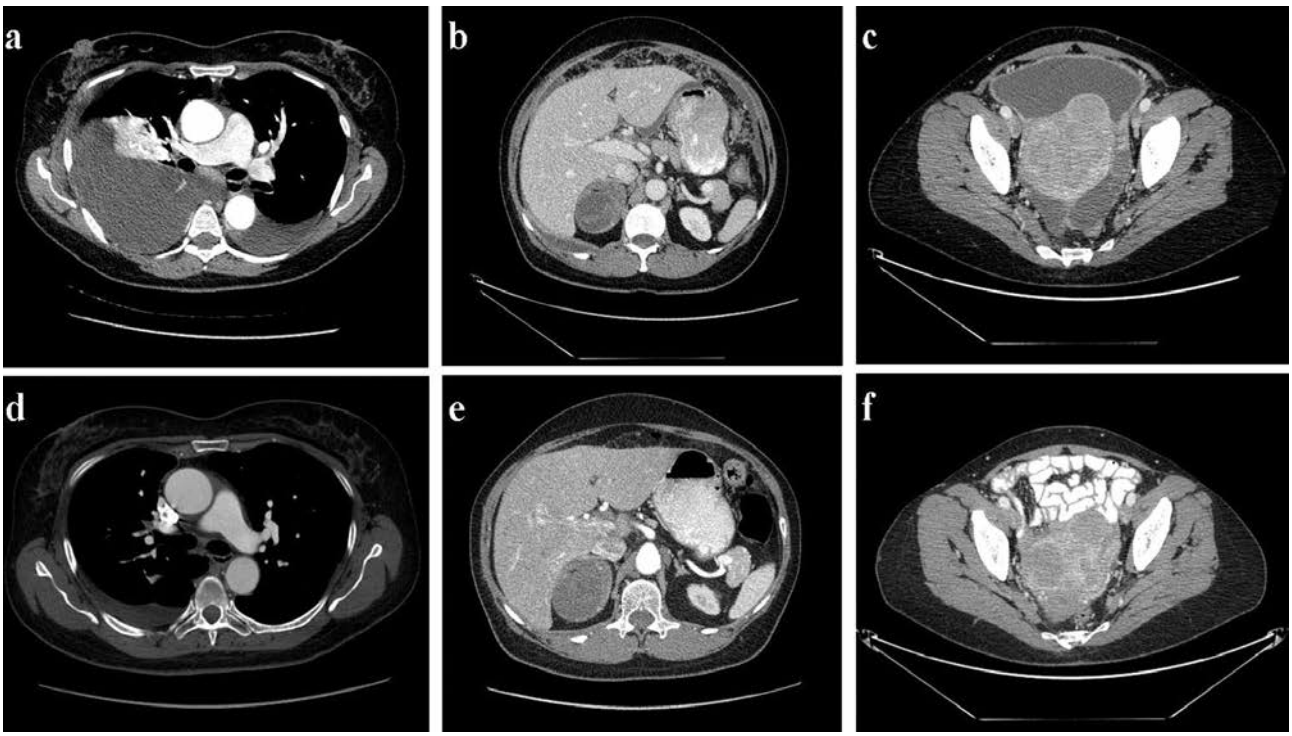

Figure 1. — A CT-scan performed July 2008, prior to chemotherapy, showing: (a) peritoneal and bilateral pleural carcinomatosis, (b) an adrenal mass of six cm and (c) pelvic mass with ascites misinterpreted as carcinomatosis. After chemotherapy, the CT-scan of November 2009 reveals: (d) bilateral pulmonary embolism, (e) unconformity of disappearance of abdominal ascites and reduction of abdominal and pleural carcinomatosis, and (f) size stability of the right adrenal mass.

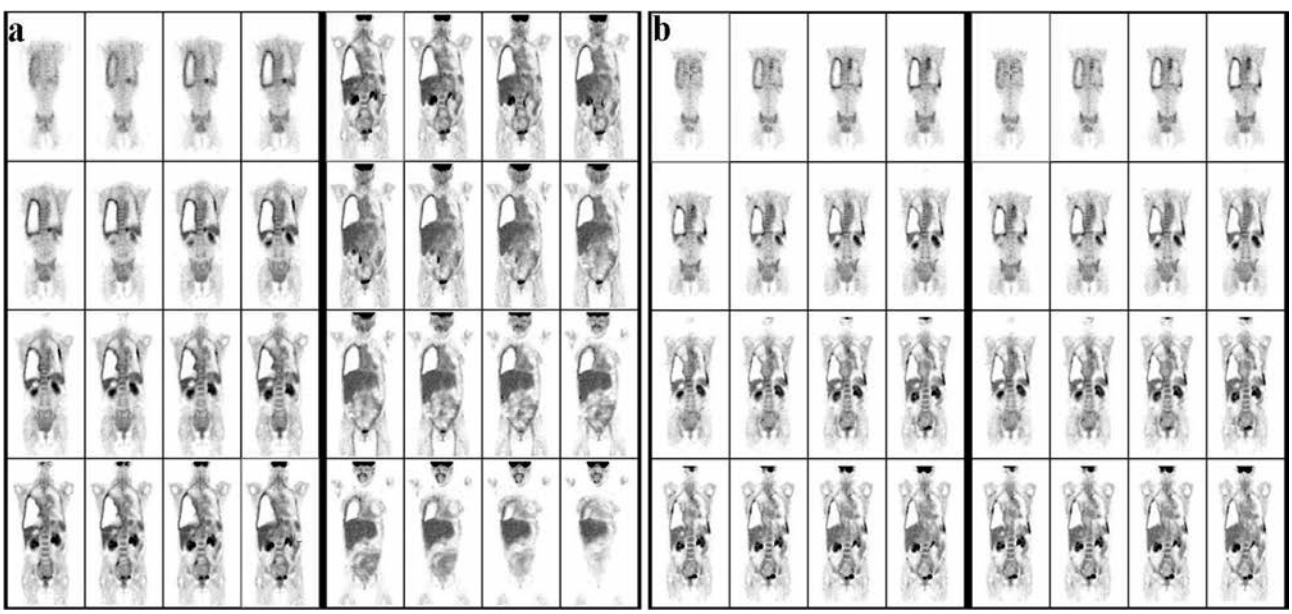

Figure 2. — (a) PET-scan taken in December 2008 showing a suspected tumor abdominal and pleural lesions. This is compatible with conclusions from the CT-scan, but no suspected fixing imagine corresponds to a right adrenal mass location. (b) PET-scan performed in February 2009 showing an evident response to anti-tubercular therapy comparatively to the one first taken in December 2008.

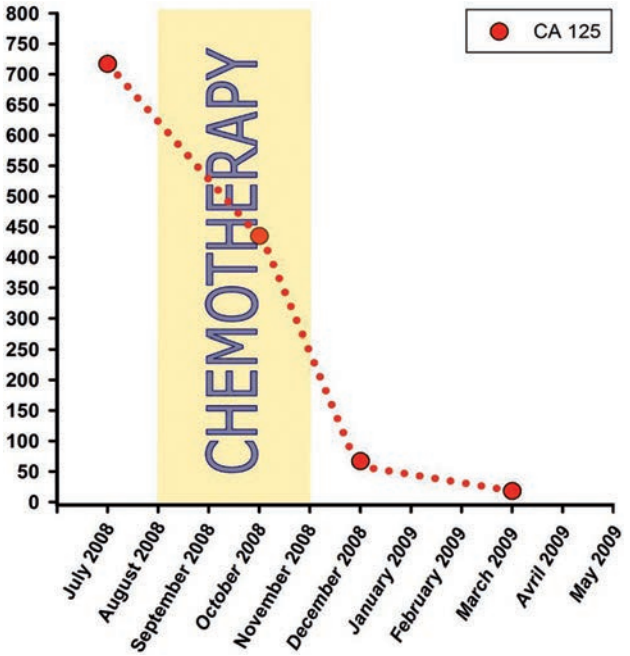

Figure 3. — Monitoring of the biological marker; CA 125 before and after chemotherapy.

As a result of the presence of pleural liquid and as a consequence of important respiratory symptomatology of the patient, it was decided to perform a pleuroscopy with biopsy in January 2009. The result of this test was surprising. No tumor cells were found, but the typical aspect of tuberculosis was revealed. Histological analysis described important necro-inflammation associated with the presence of numerous epithelioid granulomas and giant-cells; the Ziehl test was negative, finally no histological evidence of malignancy was identified. In light of this new diagnostic, an anti-tuberculosis treatment was initiated in February: isonazide 5 mg/kg, rinfapicine 9.6 mg/kg, ethambutol 15 mg/kg, and pyrazinamide. Therefore before the beginning of anti-tubercular treatment, the breath symptomatology improved . A PET-scan performed in February 2009 showed an evident response to anti-tubercular therapy comparatively to the one first made in December 2008 (Figure 2b).

To investigate the right adrenal mass, a study was performed: potassium 3.6 mmol/L, renin 995 µU/l, urinary aldosterone 144 pmol/L, hypercortisolism (cortisol at 8h 342 nmol/L and 12h 362 nmol/L) with urinary cortisol 936 nmol/24h, ACTH 4.1 ng/l, and DOC and 18-OH-progesterone were normal. In May 2009, the authors decided to precede with a adrenalectomy that confirmed a hypothesis of adrenocorticol primitive neoplasm. Furthermore, the abdominal exploration confirmed the absence of peritoneal carcinomatosis. After adrenalectomy and anti-tuberculosis therapy, the clinical status of the patient improved with reduction of pleural liquid.

Discussion

In this case, the critical condition of the patient associated with atypical cells present in cytological ascitic liquid motivated rapid initiation of chemotherapy. The fact that the patient initially responding to this treatment with improvement, was an additional aspect that led to the erroneous diagnosis of abdominal carcinomatosis. The decrease of clinical symptoms and of CA 125 after chemotherapy was misinterpreted as confirmation of peritoneal carcinosis. The authors did not observe a decrease in neutrophils during cytotoxic chemotherapy because the patient had received routine prophylaxis with GCSF: peritoneal tuberculosis of the patient has not worsened.

The authors believe that carboplatin and paclitaxel were efficient against adenocorticocarcinoma [6-8]. Thus, immunodeficiency related to adrenal carcinoma was probably reduced, possibly allowing greater local control of peritoneal tuberculosis.

Conclusion

This case reinforces the principle of histological diagnosis before beginning chemotherapy. The imaging, was not sufficient and can lead to therapeutic errors. Finally, this case illustrates that for certain types of peritoneal carcinomatosis associated with mycobacterium tuberculosis, disease progression is slow and can be better controlled for several months in case of efficient therapy and in situations favoring the tuberculosis.

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Vulvar melanoma presenting as postmenopausal bleeding: a case report

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Summary

Primary melanomas of the vulva are extremely rare, creating obstacles in the differential diagnosis of other epithelial and non-epithelial malignancies. Due to their rarity, there are only approximately 250 cases reported in the current literature. Vulvar melanomas tend to relapse locally, as well as develop locoregional and distant metastasis through lymph node and haematic dissemination. The authors describe a case of an 84-year-old Caucasian female patient, presenting with postmenopausal bleeding, consistent with primary vulvar melanoma cause, which was successfully diagnosed and treated accordingly.

Key words: Vulvar melanoma; Chemotherapy; Radiotherapy.

Introduction

Primary vulvar melanomas represent rare entities in the current bibliography. They account for 7% to 10% of all vulvar malignancies [1, 2]. Predisposing factors, such as ulcer formations, former local radiation, presence of HPV lesion, diabetes mellitus or immunosuppression, play without any doubt a very important role. Among all the prognostic factors, tumor size, depth of the invasion, lymphatic status, and grading, affect the therapeutic management [3]. The treatment of choice, regarding all melanotic lesions, especially those in the vulvar region, remains a surgical one. In most cases, wide excision with two- to three-cm margins may replace the traditional radical vulvectomy. On the other hand, in cases of lymphatic infiltration, a bilateral inguinal femoral lymphadenectomy should be considered. We must never forget the role of the sentinel node biopsy in order to avoid the lymphatic dissection. In locally advanced cases potentially requiring an extra-radical management, radiation therapy alone or together with immunotherapy remains a valuable approach.

Case Report

An 84 year-old Caucasian woman (gravida5, para5) presented with small amount of fresh bleeding, noted on tissue paper while patient was wiping herself. This was associated with pain or trauma. The patient had no history of diabetes, no family history of endometrial, colorectal or hereditary non-polyposis colorectal cancer, and she was not taking any exogenous hormones. Her last smear was at the age of 65 and it was normal. During the aforementioned bleeding period, she did not notice any discoloration or ulceration in the external genitalia, neither any lump in the lower abdomen, nor fever or bladder symptoms or significant weight loss. The physical examination revealed the presence of a black raised lesion, around 3×2 cm with irregular and distinct borders at right labium minus and satellite lesions at left labium. The lesions were non-tender, but bled on touch (Figure 1). According to the above physical findings, a biopsy became mandatory. The histological examination revealed a mucosal malignant melanoma, without B raf-gene (BRFA) V 600 mutation. The CT of the thorax/abdomen/pelvis revealed no evidence of metastatic lesions. The bone window setting described mild degenerative changes throughout the lumbar spine. The patient underwent radical anterior vulvectomy and bilateral inguinal-femoral lymphadenectomy. The histolologic report revealed the presence of ulceration, mitotic figures 26 per mm^2, Breslow's thickness of 3.0 mm, no signs of regression or lymphovascular/perineural invasion or microsatellites. The resection margins were 3.5 mm in situ component and the depth was 14 mm. According to American Joint Commission on Cancer (AJCC) TNM staging system, the lesion was T3bNxMx.

Postoperatively, the patient underwent cycles of radiotherapy. During her follow up (ten weeks postoperatively) the physical examination did not reveal any signs of regression. On the other hand, a 2×2 cm palpable lymph node at the right inguinal region was discovered. The ultrasound examination described enlarged nodes 2×2 cm at the right groin area, with increased vascularity, consistent with nodal recurrence. The thorax/abdominal/pelvic CT revealed multiple liver metastasis (largest lesion 18 mm) and two metastatic lymph nodes (4×3 cm) in the right inguinal region (Figure 2). The patient underwent cycles of palliative chemotherapy, followed by cisplatin, carboplatin, and paclitaxel. Unfortunately, she died within the first year.

Discussion

Malignant melanoma of the vulva represents the second most common malignancy of the vulva accounting for a median rate of 8.5% of all melanoma cases.[4] According

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Figure 1. — Primary melanoma of the vulva.

Figure 2. — Abdominal CT showing the liver metastasis 'A', with the arrow pointing to the lesion.

to the current literature, the five-year survival rates for the vulvar melanoma ranges from 20% to 56% [5]. The present report confirms the overall poor prognosis for vulvar melanomas as noted by Jaramillo *et al.* [6]. Over a 30-year period (1973 to 2003), there were only 644 cases of vulvar melanoma identified within Surveillance Epidemiology and End Results (SEER) database of the U.S. National Cancer Institute (NCI) [7]. According to recent studies, the majority (> 85%) of the patients, expressing vulvar melanoma, are Caucasian [8]. In general though, it is very difficult to describe the race distribution. Therefore, more studies should be conducted in the future. Imaging techniques are essential for the initial evaluation of the lesion. Pelvic MRI can provide important information regarding the extension of the local infiltration and can help in the therapeutic mapping. In order to distinguish metastatic lesions, a multidetector CT or PET/CT is mandatory. Despite the surgical and adjuvant therapy, many cases of vulvar melanomas are treated by antiangiogenic therapy [9]. Many clinical trials suggest the provision of adjuvant interferon-alpha (IFN-a) regarding the increase of recurrence free survival, but this seems not to affect the overall survival. Vulvar melanomas express higher recurrence rate in comparison with other cutaneous or mucosal melanomas. The recurrence rate is approximately 60% [10]. Classical agents concerning doses of adjuvant therapy consist of platinum/taxane regimens with a response rate of 20% [11]. New and promising agents targeting the T-cell stimulation represent the future management options [12].

Conclusion

According to the recent bibliography, vulvar melanomas represent rare entities. In many cases the prognosis and the overall survival rates are poor. Multidisciplinary cooperation in order to establish the ultimate management mapping becomes mandatory.

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Pure non-gestational choriocarcinoma arising in the ovary

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Summary

Non-gestational choriocarcinoma (NGCO) is a rare primary ovarian cancer with poor prognosis. It is important to distinguish it from gestational ovarian choriocarcinoma (GCO), because there are different treatment options. However, it is difficult to distinguish the two types by routine histologic, ultrastructural, or immunohistochemical examination. The authors present NGCO in a 41-year-old woman, which was confirmed by DNA polymorphism analysis. All tested microsatellite markers had identical DNA profiles with the same allelic sizes between tumor and normal myometrium of the patient, indicating that both tissues originated from the same person. The results confirmed that the tumor was non-gestational in origin. Although the tumor was large, the authors performed hand-assisted laparoscopic surgical (HALS) staging. After three cycles of combination chemotherapy and surgery, the patient has not had any evidence of disease 48 months after treatment. This case demonstrates the usefulness of HALS staging and DNA polymorphism analysis in NGCO.

Key words: DNA polymorphism analysis; Hand-assisted laparoscopy; Non-gestational choriocarcinoma; Ovary.

Introduction

Pure ovarian choriocarcinoma is one of the extremely rare ovarian cancers, which is classified as either gestational or non-gestational origin [1-3]. Gestational choriocarcinoma (GCO) contains at least one paternal compliment in the genes, but non-gestational choriocarcinoma (NGCO) arises from an ovarian germ cell tumors or epithelial tumor. NGCO can consist of pure trophoblasts or be mixed by germ cell tumor, containing immature teratomas, dysgerminomas, and polyembryomas. Thus, NGCO contains no paternal contribution to the genome [4]. NGCO is known to be resistant to single-agent chemotherapy and has poor prognosis compared to GCO [5]. Although it is important to distinguish each type of choriocarcinoma, it is difficult to distinguish between the two types by routine histologic, ultrastructural, or immunohistochemical examination in post-menarche women [1, 2]. A few cases have been reported where DNA polymorphism analysis was utilized to diagnose NGCO. Recently, the laparoscopic approach in early-stage ovarian cancer has markedly improved [6]. In this paper, the authors report a case of NGCO diagnosed by DNA polymorphism analysis after hand-assisted laparoscopic surgical (HALS) staging and the long-term clinical outcome.

Case Report

A 41-year-old woman with gravida 2, para 2 was admitted to Ulsan university hospital because of solid adnexal mass and vaginal bleeding of during one month prior. She did not have remarkable medical history but underwent two cesarean sections. Vital signs and laboratory findings were unremarkable. The authors checked tumor markers for malignancy evaluation. It showed that the serum beta-human chorionic gonadotropin (β-hCG) titer was increased at 1,400 mIU/ml (normal range, 0-3 mIU/ml). Other tumor markers were not remarkable; alpha fetoprotein (AFP) was 2.1 ng/ml (normal < 20 ng/ml), carcinoembryonic antigen (CEA) was 1.3 ng/ml (normal < 2 ng/ml), carbohydrate antigen 125 (CA125) was 13.9 U/ml (normal < 45 U/ml), and carbohydrate antigen 19-9 (CA19-9) was 8.3 U/ml (normal < 37 U/ml). Transvaginal sonograms and abdominopelvic computed tomography (APCT) showed a large well-capsulated round mass (109.3×98.6 mm) arising from the right adnexa (Figure 1-A). The large mass was suspected to be a malignant tumor based on radiologic reports. Chest CT, mammography, duodenofibroscopy, and colonoscopy were performed but there were no meaningful findings. The endometrial biopsy was performed and a diffuse decidual reaction was reported. For the preliminary diagnosis of ovarian cancer, she underwent HALS staging operation. A 12-mm trocar for telescope was inserted through the umbilicus and two five-mm trocars were inserted on LLQ and RLQ areas. A four-cm transverse incision was made on the suprapubic area, where fasciotomy was performed, and peritoneum was incised. A flexible wound retractor was inserted through the extended suprapubic incision. The abdomen and pelvis were carefully explored and palpated using laparoscopic vision and direct palpation, respectively. During laparoscopic exploration, the tumor was observed to be well-encapsulated in the pelvic area and there was no evidence of

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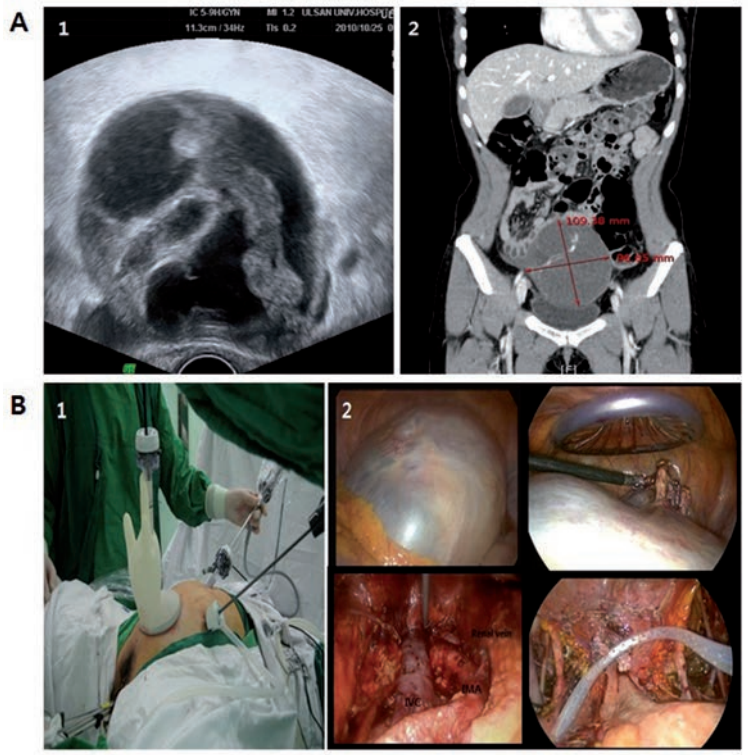

Figure 1. — A) Imaging study of non-gestational choriocarcinoma arising in the ovary. Transvaginal sonogram (A-1) and abdomino-pelvic computed tomography (A-2). A 11-cm well-defined complex cystic tumor arising from right adnexa; multiple uneven internal septa with a focus of small solid component B) Surgical view of hand-assisted laparoscopic surgical staging (HALS): preparation view (B-1) and laparoscopic view (B-2).

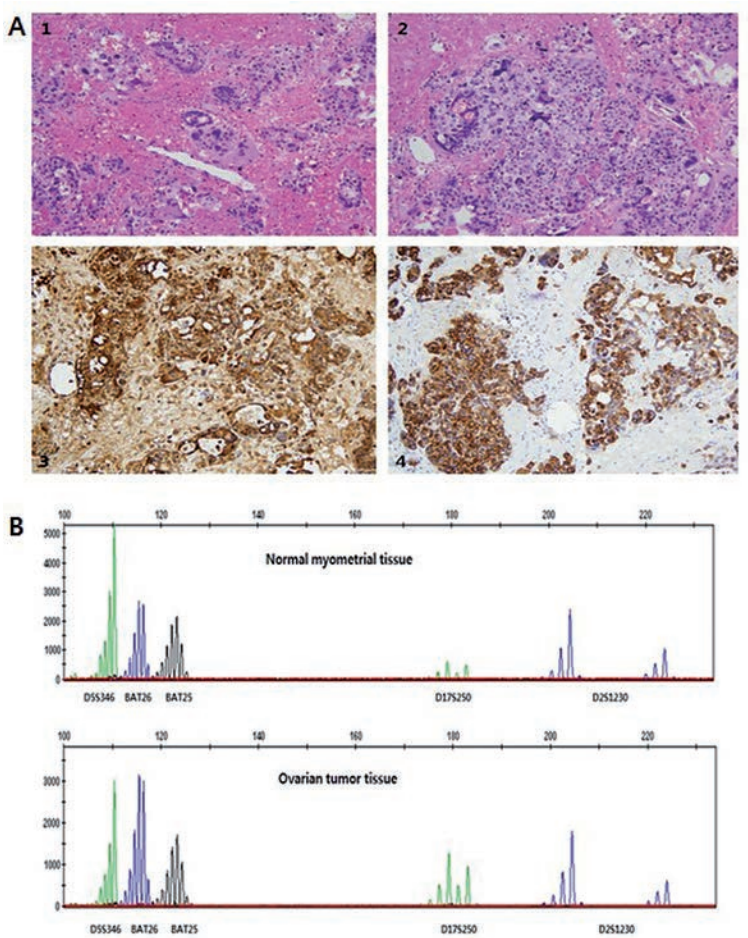

Figure 2. — A) Microscopic findings of non-gestational choriocarcinoma arising in the ovary showing a biphasic arrangement of syncytiotrophoblastic (A-1) and cytotrophoblastic tumor cells (A-2) (H&E ×200); strong immunopositivity for β-hCG in the syncytiotrophoblastic component (A-3; β-hCG immunostain ×200); positive immunostain for cyto- keratine in the tumor cell (A-4; cytokeratine immunostain ×200).
B) DNA microsatellite markers profile comparison between normal myometrium and tumor tissue. The patient was homozygous for three markers (D5S346, BAT26, and BAT25) and heterozygous for two markers (D17S250 and D2S123) in both the tumor and normal myometrial tissues. Note that the DNA profiling patterns of the normal and tumor tissues were identical at all markers tested, indicating that both tissues were from the same person. Thus, this tumor was of a non-gestational origin.

metastasis. After right salpingo-ophorectomy was performed, the specimen was removed through a wound retractor while using an endoscopic pouch to prevent the spillage of tumor content. The frozen section was suspected to be a type a choriocarcinoma. The authors performed laparoscopic assisted vaginal hysterectomy (LAVH), left salpingo-ophorectomy, omentectomy, pelvic and para-aortic lymph node dissection, appendectomy, and multiple biopsies (paracolic gutter and pelvic peritoneum). Optimal debulking was achieved with no macroscopic residual tumor (Figure 1-B).

Microscopically, the tumor was composed of pleomorphic biphasic arrangement with cytotrophoblasts and syncytiotrophoblasts admixed with hemorrhage and necrotic tissue. The syncytiotrophoblasts were strongly immunopositive for β-hCG and cytokeratin staining (Figure 2-A). The tumor was intact with the ovarian surface.

DNA profiling was performed between normal myometrium and ovarian tumor to determine the genetic origins of the choriocarcinoma. The authors used selected a consensus panel of five microsatellite markers: BAT25, BAT26, D5S346, D17S250, and D2S123. These markers are highly polymorphic microsatellite markers used for diagnosing hereditary non-polyposis colorectal cancer (HNPCC). The authors conducted multiplex polymerase chain reaction (PCR) for markers with different amplification sizes and analyzed the results using the GeneScan 3.1 software package. The present patient was homozygous for three markers (D5S346, BAT 26, and BAT25) and heterozygous for two markers (D17S250 and D2s123) in both normal myometrial tissue and the tumor. DNA profiling patterns showed the same allelic sizes of all tested microsatellite markers between tumor and myometrial tissues (Figure 2-B). As a result, a pure NGCO of the ovary, Stage Ia (FIGO staging system) was confirmed in pathological reports.

Postoperatively, the patient was treated with bleomycin, etoposide, and cisplatin (BEP) adjuvant combination chemotherapy every 28 days for three cycles. The serum β-hCG level decreased to 28.7 mIU/ml five days after surgery, and was within the normal range after one cycle of chemotherapy. The patient recovered well after surgery and was treated with three cycles of chemotherapy without any complications. The patient has shown no signs of recurrence 48 months after completing chemotherapy.

Discussion

Primary ovarian choriocarcinoma is a very rare type of ovarian cancer, with an incidence rate of 0.008 per 100,000 women-year in United States [7]. Ovarian choriocarcinoma is classified based on its origin as gestational or non-gestational. GCO arises from an ovarian pregnancy or metastasis from regressed or occult primary gestational choriocarcinoma in the genital tract, as in the uterus [3, 8]. NGCO arises from germ cell differentiation in trophoblast structures and coexist with other malignant germ cell components [3, 8]. In general, GCO is treated with a single chemo-agent but NGCO is treated with regimen of multiple chemo-agents, including vincristine, actinomycin-D, cyclophosphamide (VAC), BEP, and etoposide, methotrexate, actinomycin-D, cyclophosphamide, oncovine (EMA-CO). Compared to GCO, NGCO is more likely to metastasize to the lymphatic system and spread within abdominal cavity, and has worse prognosis [8]; however, Lanzhou *et al*. suggested that the five-year survival rate was 79.4% in patients who underwent adjuvant multiple drug chemotherapy [9]. Bao *et al.* reported that the prognosis of NCGO is related with whether or not they received adjuvant multiple drug chemotherapy [2]. Here, the authors report a patient with NCGO who was treated with BEP regimen after curative surgery and has had a disease-free survival of 48 months.

DNA polymorphism analysis is a useful method to distinguish between GCO and NGCO [10]. However, a review of the literature only found five cases where DNA polymorphism analysis was used to diagnose NGCO (Table 1).

NGCO can be diagnosed with histological finding in patients who are sexually immature, who have never had sexual intercourse, or are postmenopausal [8]. However it is difficult to differentiate the two subtypes by histological finding, particularly in patients of reproductive age and in pure choriocarcinoma tumors without the other components of germ cell tumors. Thus the DNA polymorphism analysis would be a more useful method in women of childbearing age. For DNA polymorphism analysis, there is no consensus as to how many microsatellite markers are needed for diagnosis. Tsujioka *et al*. reported that they could diagnose NGCO using only two or three microsatellite markers [10]. Yamamoto *et al*. suggested that DNA analysis using more microsatellite markers facilitates a more accurate diagnosis, so they performed DNA analysis with 15 loci [11]. Koo *et al*. assayed eight microsatellite markers for identification of the tumor origins [3]. In this study, DNA polymorphism analysis was performed using three homozygous markers (D5S346, BAT26, and BAT25) and two heterozygous markers (D17S250 and D2S123) in both the tumor and normal myometrial tissues. These five markers were reliable for evaluating the origin of the primary tumor. Thus, the present authors suggest that five markers for DNA analysis may be a reliable number for diagnosis of NGCO. Further consensus for how many markers are needed to accurately diagnose NGCO are needed.

In a comparative study between laparoscopic and laparotomic approach for early-stage ovarian cancer, Ghezzi *et al.* reported that there was no significantly difference in postoperative complications, upstaging effects, appropriate lymph node retrieval, disease-free survival, and overall survival between the two different approaches. They recommended a laparoscopic approach for the standard surgical staging for early-stage ovarian cancer [6]. In 28 cases of laparoscopic approach for early-stage malignant non-epithelial ovarian tumors, Shim *et al.* reported that laparoscopic approach could achieve good results, even for tumors greater than five cm and those that require aggressive lymph node dissection [12]. In the present study, the ovarian tumor featured was well-encapsulated, intact, large (about 11cm), and there was no sign of metastasis based on preoperative radiologic findings. The present authors performed HALS staging operation for the patient. They

Table 1. — *Details of 48 Cases of non-gestational choriocarcinoma of ovary (our case included).*

	Author	Age (years)	Chief complaints	Tumor Characteristics	Tumor side	Surgery	Stage	hCG (min/mL)	Diagnostic method	Chemotherapy regimen	Outcome
1	Simard 1937	17	NS	NS	Right	RO	IIB	Elevated	History, histology	None	DOD 4 months
2	Backus & Griffin 1941	13	NS	NS	NS	TAH BSO	IIB	NS	History, histology	None	DOD 6 months
3	Oliver & Horne 1948	11	Pain in abdomen	Large, necrotic hemorrhagic	Right	RSO	IIB	Elevated	History, histology	None	DOD 4 months
4	Groeber 1963	13	NS	NS	Left	LO	IA	NS	History, histology	None	DOD 4 months
5	Dehaan 1965	7	Pain in abdomen	12cm	Right	RSO	IA	200	History, histology	None	NED 19 months
6	Hay & Stewart 1969	13	Pain in abdomen	8cm, necrotic hemoperitoneum	Right	RSO	IC	Elevated	History, histology	MTX	NED 15 months
7	Panayotou et al. 1971	12	Pain in abdomen	10.5cm, necrotic hemorrhagic	Left	TAH BSO	I	Elevated	History, histology	MTX	DOD 5 months
8	Smith et al. 1973	7	NS	NS	NS	YES, NS	NS	NS	History, histology	MAC	NED 8 months
9	Shah et al. 1974	14	Lump in abdomen	25cm, necrotic hemoperitoneum	Right	Autopsy	II	NS	History, histology	NS	DOD
10	Adelman et al. 1975	1	NS	NS	NS	USO	NS	NS	History, histology	MAC	NED
11	Gerbie et al. 1975	16	NS	NS	Left	LSO	IV	NS	History, histology	MTX	NED 11 years
12	Gerbie et al. 1975	17	NS	NS	Right	RO	IA	NS	History, histology	MTX Act-D	NED 6 years
13	Gerbie et al. 1975	17	NS	NS	Left	LSO	III	NS	History, histology	MAC	NED 1 year
14	Stevens et al. 1979	19	Pain in abdomen	Large	Left	TAH BSO	IV	160 (urine)	History, histology	MTX Act-D	DOD 10 days
15	Piver & Lurain 1979	NS	NS	NS	NS	NS	IC	Elevated	History, histology	MTX Act-D	NED 8 months
16	Creasman et al. 1979	NS	NS	NS	NS	BSO	III	NS	History,histology	MAC	NED 54 months
17	Vance & Greisinger 1985	9	Pain in abdomen	14cm, friable hemoperitoneum	Right	RO	IC	Elevated	History, histology	PVB	NED 6 months
18	Axe et al. 1985	6	Vaginal bleeding	Friable, hemoperitoneum	Right	RO	IC	NS	History, histology	None	NED 10 years
19	Axe et al. 1985	11	Pain in abdomen	Friable, hemorrhagic	Right	RO	I	Elevated	History, histology	None	DOD
20	Raju et al. 1985	16	Fever, dysuria	18cm, necrotic hemoperitoneum	Right	Autopsy	IV	NS	History, histology	NS	DOD
21	Sengupta & Everett 1987	11	NS	NS	NS	UO	NS	NS	History, histology	NS	NS
22	Pippitt et al. 1988	NS	NS	NS	NS	UO	NS	NS	History, histology	VAB-VI	NED 9 months
23	Spingler et al. 1990	20	Pain in abdomen	Ruptured tumor	NS	YES, NS	NS	NS	History, histology	NS	DOD
24	Gribbon et al. 1992	NS	NS	NS	NS	YES, NS	NS	Elevated	History, histology	NS	DOD 4 months
25	Gribbon et al. 1992	11	NS	NS	NS	YES, NS	IIC	Elevated	History, histology	NS	NED 1 year
26	Brown et al. 1993	11	NS	NS	NS	USO	NS	NS	History, histology	NS	NED 32 months
27	Trigueros et al. 1995	21	NS	24cm	Right	TAH BSO	III	200,000	History, histology	PVB	NED 4 years
28	Arima et al. 1995	26	NS	NS	NS	NS	NS	NS	DNA analysis	NS	NS
29	Arima et al. 1995	21	NS	NS	NS	NS	NS	NS	DNA analysis	NS	NS
30	Chou et al. 1997	39	NS	20cm	Left	TAH BSO	IV	71,885	History, histology	Carboplatin Etoposide Ifosfamide	NED 17 months
31	Gungor et al. 1999	16	Pelvic pain	NS	NS	TAH Bilateral ovarian wedge resection	NS	20,000	History, histology	EMA-CO	DOD 6 months
32	Inaba et al. 2000	12	Irregular Bleeding	11cm, ruptured	Right	RSO LOC	III	25,000	History, histology	PEB(high dose)	NED 3 years
33	Goswami et al. 2001	18	Pain in abdomen	10x12cm, friable	Left	LSO ROC	IA	88,385	History, histology	MAC	NED 5 months
34	Tsujioka et al. 2003	19	Pain in abdomen	9cm	Left	LSO	IV	200,000	DNA analysis	EMA-CO	NED
35	Ozdemir et al. 2004	13	Pain in abdomen	7x8cm, ruptured	Right	RSO	NS	91,028	History, histology	MAC	NED 9 months
36	Corakci et al. 2005	22	Pain in abdomen	8cm	Right	RSO	IA	15,050	History, histology	PEB	NED
37	Koo et al. 2006	33	Vaginal bleeding	10x14cm, ruptured		TAH BSO	NS	185,000	History, histology	MAC	NED 18 months
38	Yamamoto et al. 2007	19	Vaginal bleeding	6.8cm	Left	LSO	IA	206,949	DNA analysis	MEA	NED 12 months
39	Roghaei et al. 2007	47	Pain in abdomen Vaginal bleeding	10cm	Left	TAH BSO partial-omentectomy	IV	970	History, histology	EMA-CO	NED 12 months
40	Bao Kong et al. 2009	10	Pain in abdomen	15x13cm, ruptured	Left	LSO	IC	7,957	History, histology	PVB	NED 2 months
41	Sung Hye Park et al. 2009	55	Pelvic pain, dry cough	6x5cm	Right	TAH BSO	IV	64,838	History, histology	BEP	NED 20 months
42	Narges Izadi Mood et al. 2009	31	Pain in abdomen	7x7cm	Right	RSO	I	1,000	History, histology	EMA-CE	NED 7 years
43	Narges Izadi Mood et al. 2009	32	Vaginal spooting	13x11cm	Left	TAH BSO infracolic-omentectomy	IIA	5,500	History, histology	BEP	NED 5 years
44	Lin Lv et al. 2011	48	Vaginal bleeding	18x15cm, friable		TAH BSO PLND PALNS omentectomy appecdectomy	III	7,664	History, histology	BEP	NED 1 year
45	Pedro Exman et al. 2013	24	General weakness	12x10cm	Left	TAH BSO omentectomy	IV	675,713	DNA analysis	Neo-adjuvant BEP	NS
46	Youn jin Choi et al. 2013	33	Pain in abdomen Vaginal bleeding	5x4cm, ruptured	Left	LO ROC peritoneal mass biopsy	III	17,763	History, histology	EMA	NED 5 years
47	Eun Jin Heo et al. 2014	12	Vaginal bleeding	4x4cm	Left	LSO partial omentectomy multiple peritoneal biopsy	IA	20,257	History, histology	BEP	NED 14 months
48	Present case	40	Vaginal bleeding	11x10cm	Right	LAVH BSO PLND PALND omentectomy appendectomy	IA	1,400	DNA analysis	BEP	NED 4 years

Note. NS, not stated; NED, no evidence of disease; DOD, death of disease. TAH, total abdominal hysterectomy; LAVH, laparoscopic assisted vaginal hysterectomy; BSO, bilateral salpingo-oophorectomy; RSO; right salpingo-oophrectomy; LSO, left salpingo-oophrectomy; UO, unilateral oophorectomy; RO, right oophorectomy; LO, left oophorectomy; ROC, right ovarian cystectomy; LOC, left ovarian cystectomy; PLND, pelvic lymph node dissection; PALNS, para-aortic lymph node sampling; PALND, para-aortic lymph node dissection; MTX, methotrexate; Act-D, actinomycin D; MAC, methotrexate, actinomycin-D, chlorambucil; VBP, vinblastine, bleomycin, cisplatin; VAB-VI, vinblastine, bleomycin, cisplatin, actinomycin D, cyclophosphamide; PEB, cisplatin, etoposide, bleomycin; EMA-CO, etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine; MEA, methotrexate, etoposide, dactinomycin; BEP, bleomycin, etoposide, cisplatin; EMA-CE, etoposide, methotrexate, actinomycin D, cisplatin, etoposid.

achieved a good surgical result without any complications, such as tumor spillage. For well-encapsulated large ovarian tumor without rupture or metastasis, a laparoscopic approach could be one of the good staging options for early stage ovarian cancer.

In conclusion, NGCO is a very rare, and more resistant to single-agent chemotherapy than that of gestational origin. Thus, it is important to differentiate between gestational and non-gestational origin using DNA polymorphism analysis for selection of chemotherapy in post-menarche women. In early-stage ovarian cancer, HALS staging may be a good treatment option for large ovarian tumors. Based on diagnosis of surgery and DNA analysis, combination chemotherapy may be important in improving the prognosis of a patient.

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Primary mucinous carcinoma of the vulva with signet ring cells deriving from the cloaca

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Summary

Vulvar neoplasias are rarely encountered lesions at female genital tract, regardless if they are primary or metastatic. Presence of signet ring cells in a tumour at female genito-urinary tract is highly suggestive of a metastatic lesion particularly from a gastrointestinal tumour. Here the authors present a case of vulvar carcinoma with signet ring cells with an undetermined primary site possibly originating from embryonic cloaca.

Key words: Signet ring cell; Vulvar tumour; Primary vulvar cancer; Cloaca.

Introduction

Vulvar tumours constitute 3-5% of gynaecologic malignancies and compose < 1% of all malignancies in women [1]. Majority of the vulvar tumours are squamous carcinomas. Vulvar mucinous adenocarcinomas are extremely rare regardless of being primary or metastatic. Primary mucinous tumours of vulva may arise from Bartholin's glands, sweat glands, sebaceous glands, mesonephric remnants, ectopic breast tissue or in association with entero-cutaneous fistulas [2, 3]. Presence of signet ring cells in a tumour at genito-urinary site is highly suggestive for a metastatic lesion possibly from a gastro-intestinal origin [4, 5]. Here the authors present a case of signet ring cell mucinous adnenocarcinoma of the vulva with an undetermined primary origin.

Case Report

A 62-years-old woman presented with spotting and with a history of two vaginal births. Her medical history was unremarkable except for total abdominal hysterectomy and bilateral salpingo-oophorectomy performed in 2001 due to uterine fibroids. Her physical examination revealed an irregularly contoured rough nodule with a diameter of three centimetres on the medial side of right labium major. Speculum examination revealed a normal appearing vaginal vault. Transvaginal ultrasonography did not demonstrate a pelvic pathology. Pap smear was normal and tumour markers including CA 125, CA 19-9, CA 15-3, CA 72-4, and CEA were within normal limits (4.4 U/ml, 3 U/ml, 16.5 U/ml, 2.28 U/ml, and 0.38 ng/ml respectively). An excisional biopsy was performed. Pathologic examination revealed a mucinous adenocarcinoma with signet ring cells. The tumour was observed to invade dermis, extending trough the epidermis, and causing micro-ulcerations on stratified squamous epithelium of vulvar skin (Figure 1). Signet ring cells observed within the mucinous lakes (Figure 2A), stained positive for mucicarmine. Surgical margins of the resected specimen were free from tumoral cells. Immunohistochemical staining was performed. Tumour cells were extensively stained with CK20 (Figure 2B), CDx2, MUC2. Focal areas of staining were observed with CK7 and MUC5AC. MUC1 GCDFP-15 stains were negative for the specimen. Further investigations were performed to detect the possible primary site of the tumour considering the staining pattern, particularly for a gastrointestinal site tumour. Colonoscopic and gastro-duedonoscopic examinations were normal. Mammography did not demonstrate any suspicious lesions. PET-CT was unremarkable except for a mildly increased uptake compatible for inflammatory response (SUV max: 1.9) at the site of vulvar biopsy. Toraco-abdomino-pelvic CT did not demonstrate any pathologic lesions but a haemangioma with a diameter of 11 mm within liver.

A decision was made to perform expectant management. Monitoring included systemic physical examination, pelvic examination, vaginal vault smear, serum tumour marker level assessment, mammography, colonoscopy, and gastro-duedonoscopy. After two years of uneventful follow-up, patient presented with erythematous swelling at right thigh and pain on right aspect of pelvis. Pelvic examination revealed a rough reddish nodule with irregular contours measuring approximately 3×4 cm. Lower extremity venous Doppler examination did not reveal any signs of deep venous thrombosis or venous insufficiency. Serum levels of CA125, CA 19-9, and CEA were found within normal range. Pelvic MRI revealed irregularly contoured mass originating from right labium major, extending to distal portion of urethra and vaginal orifice, and infiltrating through pelvic floor muscles. Right para-iliac and inguinal lympadenopathy with subcutaneous edema in favour of extra-capsular lymphatic spread and nodular lesions at right iliac bone and right lateral side of sacral bone indicating metastasis were also demonstrated by pelvic MRI. These findings were considered as a recurrence of previously diagnosed malignancy and chemotherapy regimen consisting in carboplatin and paclitaxel was started. After six cycles of chemotherapy, PET-CT imaging demonstrated a physiological spread of F-18 FDG throughout the body. Colonoscopic, gastro-duedonoscopic examinations, mammography, and serum tumour marker levels were normal as well. The pa-

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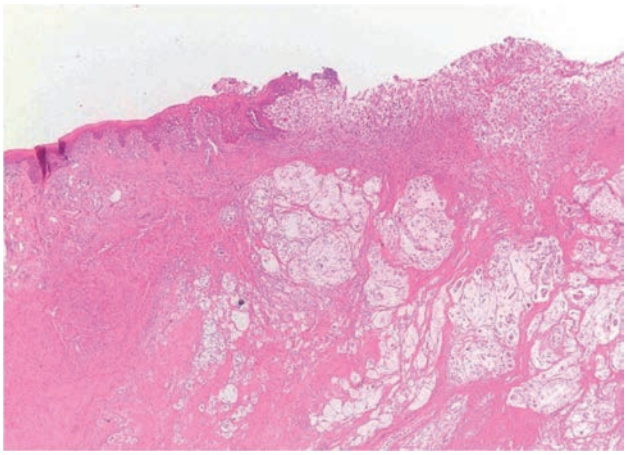

Figure 1. — Infiltration of tumour cells in stratified squamous epithelium causing ulceration.

tient is disease-free at 41 months after the diagnosis and 12 months after completion of the chemotherapic regimen.

Discussion

Metastatic tumours of the vulva constitute only 5-8% of all vulvar malignancies. Vulvar metastases generally indicate a widespread primary disease and are usually considered as a preterminal event. The duration of survival changes in cases with metastatic vulvar lesions is in respect of the primary malignancy. Majority of these cases are reported to have disseminated diseases when diagnosed to have vulvar metastases. The overall mean survival rate of the women that have malignancies with vulvar metastases was estimated as 35.6 months subsequent to the diagnosis of metastatic vulvar lesions [1]. Most common extra-genital primary sites of vulvar metastasis were reported as breast carcinoma and gastro-intestinal system tumours [6]. In this case, any possible primary site of vulvar tumour was unable to be demonstrated despite all attempts. Systemic physical examinations, pelvic examinations, serum tumour marker levels, vaginal vault smears, PET-CT, mammographies, colonoscopies, and gastro-duedonoscopies were all normal at initial investigation, as well as the follow-up visits even at the time of recurrence that was occurred two years after the local excision.

Primary mucinous carcinomas of the vulva may arise from ectopic breast tissue, Bartholin's glands, sweat glands, sebaceous glands, mesonephric remnants or in association with entero-cutaneous fistulas [2,3].

Normally vulva includes some mammary-like tissue as a derivative of milk-lines. However, there are few number of reported adenocarcinoma cases derived from this ectopic mammary tissue. These usually form glandular structures and exhibit oestrogen or progesterone receptor positivity [7-10]. Although signet ring cells could be seen in breast cancer in rare cases [11] immunohistochemical features of the present case were not consistent with a carcinoma derived from breast tissue.

Primary carcinoma of Bartholin's gland constitutes 2-7% of all vulvar malignancies and adenocarcinomas comprise nearly 40% of Bartholin's gland carcinomas [12]. There are some criteria described for the definitive diagnosis of Bartholin gland carcinoma, such as demonstration of transition from normal Bartholin gland tissue to neoplastic tissue, histologically compatible localization of tumour with the origin of Bartholin's gland, and no evidence of other primary tumour [13]. In the present case there was no transition observed between normal Bartholin gland tissue and neoplastic tissue. Tumour margins were not connected with Bartholin's gland.

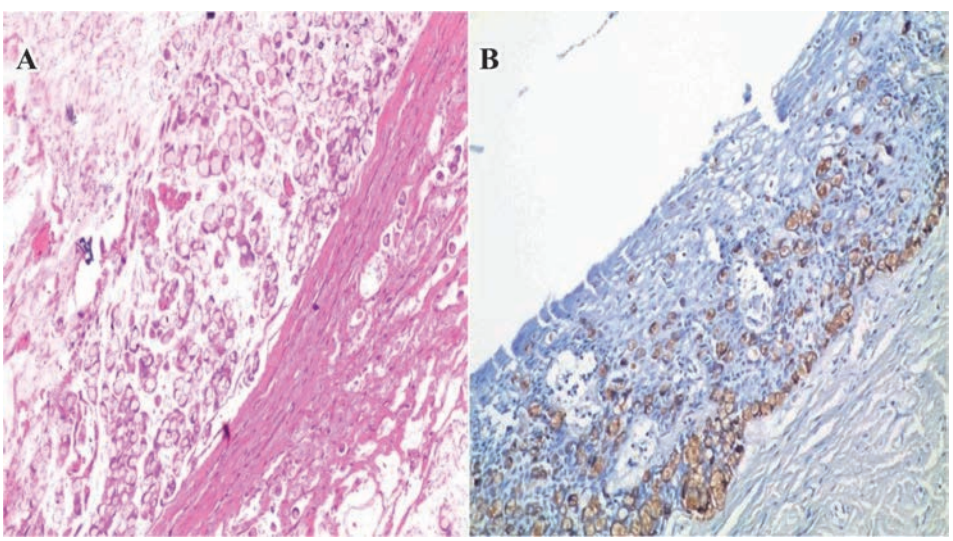

Figure 2. — A) Epithelial cells appearing as signet ring cells within the mucinous lakes. B) Staining of tumour cells with CK20 reside within the mucinous lakes and spread through the epidermis.

Table 1. — *Summary of the clinical presentation, management, and outcomes of reported vulvar adenocarcinoma cases with cloacogenic origin*

Author	Age (years)	Vulvar lesion	Other clinical findings	Treatment	Inguinal-femoral LNM	Distant metastasis	Prognosis
Tiltman et al. [15]	50	NA	NA	Modified RV+ BL IFLND	Yes	No	12 months DFF
Kennedy *et al.* [16]	Case 1: 54 Case 2: 63	NA	NA	Case 1: RV+BIFLND Case 2: Wide LE	No	No	Case1: 120 months Case 2: 48 months DFF
Ghamande *et al.* [2]	67	1.2 cm	No	RV+BIFLND	No	No	17 months DFF
Willen *et al.* [17]	57	1 cm	No	Wide LE	No	No	26 months DFF
Zaidi *et al.* [18]	43	4 cm	No	Modified RV+BILND	No	No	18 months DFF
Rodriguez *et al.* [19]	69	1,5 cm	No	Wide LE	-	No	36 months DFF
Liu *et al.* [20]	49	1.8 cm	Inguinal LAP	Wide LE+ BILND	No	No	24 months DFF
Dubé *et al.* [21]	58	2 cm	No	RHV+ ULIFLND	No	No	16 months DFF
Cormio *et al.* [22]	Case 1: 58 Case 2: 42	NA	NA	Case 1: RHV+BLIFLND Case 2: RV+BLIFLND	No	No	Case 1: metastatic colon cancer 36 months after treatment Case 2: 39 months DFF + dysplastic polyp in sigmoid colon
Karkouche *et al.* [23]	31	NR	NR	LE (recurrence after 6 months) LE for recurrence	-	-	15 months DFF
Chibbar *et al.* [24]	49	1 cm (multiple)	Inguinal LAP+ lower vaginal involvement+ lung metastasis	Chemo-radiation Punch biopsy+BLIFLND	Yes	Yes	DOD 27 months later
Musella *et al.* [25]	57	5 cm	Inguinal LAP+ Lower vaginal involvement	Neoadjuvant CT Radical vulvectomy+ ULIFLND	Yes	No	4 months DFF
Present case	62	3 cm Recurrence: 4 cm	No Recurrence: extensive spread	Wide LE Recurrence: chemotherapy	- Recurrence: yes	No Recurrence: yes	Recurrence after 24 months 12 months DFF after recurrence

RV: radical vulvectomy; RHV: radical hemivulvectomy; LE: local excision; LAP: lymphadenopathy; LNM: lymph node metastasis; BIFLND: bilateral inguinal-femoral lymph node dissection; ULIFLND: unilateral inguinal-femoral lymph node dissection; BILND: bilateral inguinal lymph node dissection; DFF: disease-free follow-up; DOD: died of disease; NA: not available; NR: not reported.

Immunohistochemical staining features of the tumour indicated that this tumour may not be derived from glandular heterotopias. In glandular heterotopias of vulva, cells stain positively for CK7, CD-X2 and CEA, but negatively for CK20 [14]. Extensive staining with CK20 in the setting of focal staining with CK7 in the present case strongly suggests that a glandular heterotopia is unlikely and the lesion might be secondary to metastasis, possibly from gastrointestinal system. Moreover tumour cells have not stained with GCDFP 15, excluding the possibility of extra-mammary Paget disease.

Primary vulvar carcinomas originating from cloacal remnants have been published in a handful of reports that are summarized at Table 1 [2, 15-25]. These tumours are known to have glandular structures, could be continuous with epidermis and may cause focal ulcerations. Signet ring cells could be observed in the tumours derived from cloacal origin, however a primary mucinous tumour of the vulva with signet ring cells derived from cloacal remnants is an exceptionally rare occurrence and has not been reported before.

The tumour in the present case might be derived from embryonic remnants of cloaca at vulvar region. On the other hand, the present patient has a history of two vaginal births with right mediolateral episiotomy and rectal mucosa could be involved in the course of episiotomy repair. In any case, origin of this tumour deemed to be closely related with cloaca.

Limited number of reports about this issue indicates a relatively indolent course and favourable prognosis in these types of tumours [24]. Less aggressive surgeries like local wide excision instead of radical vulvectomy could be curative in these tumours, particularly in the cases with negative surgical margins [16, 17] and in the absence of any clinical suspicion for metastasis to regional lymph nodes or distant sites.

Cormio *et al.* [22] reported two cases of vulvar cancer with cloacogenic origin. One of these cases was found to have disseminated colon cancer 36 months after surgical treatment of vulvar disease and the other case was found to have a dysplastic polyp at colon 39 months after vulvar surgery. The present authors have performed colonoscopy and gastro-duedonoscopy at follow-up visits. In their opinion, dissimilar with other types of vulvar cancer, performing colonoscopy and gastro-duedonoscopy in primary cloacogenic vulvar tumor follow-up, seems reasonable unless otherwise proven by future studies.

In the present case, the malignancy had relapsed after two years. Invasion of pelvic floor muscles, involvement of lymph nodes with extra-capsular spread and pelvic bone metastasis were detected. These factors indicate an unfavourable prognosis. This recurrence could be classified as FIGO Stage IVA if considered to be a primary vulvar tumour. However, despite these unfavourable prognostic factors, the disease seems to respond unpredictably well to carboplatin-paclitaxel regimen, similar to the case reported by Musella *et al.* [25]. A vulvar tumour with > two cm diameter and confined to the vulva or perineum was classified as $T_{1b}N_0M_0$ according to TNM classification and all patients with tumours larger than two cm require a thorough inguinal-femoral lymphadenectomy [26]. However a thorough inguinal-femoral lymphadenectomy is associated with long-term morbidities and should be avoided whenever the survival will not be compromised by omission of this procedure. Depending on the scarce data about this rare occurrence, considering the indolent course of this tumour, and the probable susceptibility to chemotherapy as indicated by limited number of reports in literature, future treatment of these patients might include more limited surgeries (for example to avoid the possible long-term morbidities associated with the groin dissection), if efficiency of a narrow surgery in preventing relapses is demonstrated to be similarly successful to wide surgeries in reliable studies that will be conducted for this aspect. However, without doubt, this issue should be clarified by studies with large number of cases prior shifting to a more conservative surgical approach and without support of reliable evidence limited surgeries should not be considered as safe and effective.

In conclusion, although the current evidence is not sufficient to reliably recommend a modality of treatment due to rarity of this kind of tumours, primary mucinous tumors of the vulva with a cloacal origin could have relatively good prognosis even if they include signet ring cells and might respond well to chemotherapy regimens comprising carbopatin and paclitaxel.

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Fast and unfavorable course of invasive cancer of the uterine cervix associated with pregnancy despite of a typical treatment. Case report of 35-year-old pregnant multipara

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Summary

Carcinoma of the cervix is the most common malignancy associated with pregnancy. The frequency of cervical cancer is estimated to range from 1/1,200 to 1/10,000 pregnancies. The symptoms of cervical cancer are not specific and can be mistaken as frequent symptoms associated with other pathologies of pregnancy. The diagnostic procedure is similar to the one which should be proposed to unpregnant women. The treatment of cervical cancer depends on gestational age. The final treatment and further prognosis is carried out after delivery. The authors present the case of a 35-year-old woman at 34 weeks of gestation diagnosed with Stage IB cervical cancer. Treatment was delayed until fetal maturity and an elective cesarean section was performed at 36 weeks' gestation, followed by a radical hysterectomy, bilateral salpingo-oophorectomy, and a pelvic lymphadenectomy. Patient underwent adjuvant radiochemotherapy and brachytherapy. Recurrence of neoplastic process was found after one year.

Key words: Cervical cancer; Pregnancy; Total hysterectomy.

Introduction

Carcinoma of the cervix is one the most common malignancy associated with pregnancy. According to the literature its frequency is estimated at approximately 1.2−10.6 cases per 10,000 pregnancies in different populations, while in Poland the frequency of this cancer is estimated from 1/1,370 to 1/2,500 of pregnancies [1, 2], in which 69−83% of invasive carcinomas associated with pregnancy is at Stage I. According to histological classification, 81−87% of them are recognised as squamous cell carcinoma and 7−15% of them are recognised as adenocarcinoma [3]. The mean age of patients suffering from cervical cancer is from 31 to 35 years. The most common symptoms of cervical cancer like spotting, and pain can be disguised by pregnancy, due to the fact that they may be interpreted as the symptoms associated with pregnancy. It is important to be aware of the fact that carcinoma of the cervix is usually asymptomatic, especially in the early stages [2, 4, 5]. For these reasons obtaining of cervical smear in pregnancy should be a routine procedure carried out during the first visit to a gynaecologist. Diagnostic procedures used for detecting cervical cancer in pregnant women are similar to those used for diagnosis of this cancer in unpregnant patients. Main diagnostic procedures include: cervical cytology, colposcopy, and biopsy [6].

Treatment of cervical cancer associated with pregnancy depends on the stage of the cancer and on the gestational age. The choice of the best time for surgery or look and wait management depends not only on the stage of pregnancy but also on the clinical and histopathological characteristics, the depth of invasion, and the grade of tumor's differentiation [2]. This decision should be made jointly by patient and her doctor. Finally the patient's choice of method of treatment must be made based on very accurate information [7]. The expedited treatment is recommended before 20 weeks of gestation, while after this time the treatment can be postponed until the fetus reaches lung maturity [4, 8]. Due to immaturity of the fetal's lungs during the first half of pregnancy, the primary treatment of Stage Ib and IIa of cancer is extended hysterectomy and lymphadenectomy. In this case the delay of the treatment might be a life-threatening situation. The primary treatment of early stages of cervical cancer in advanced pregnancy is a cesarean section accompanied by expedited adjuvant therapy - the Wertheim-Meigs extended hysterectomy combined with radiotherapy.

According to current state of research on cervical carcinoma, planned delay of treatment does not worsen the prognosis. At present it does not seem to be confirmed that pregnancy is causing the progression of the disease [9]. However, the decision about the delay of treatment until the fetus is potentially able to live outside the uterus is usually made by the patient. During this time pregnant woman

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remains under strict observation in order to detect possible progression of the cancer and to institute the treatment after taking into account the level of fetal lung maturity [2].

The prognosis in cervical cancer in pregnant women is not much different from that in unpregnant patients and it mainly depends on the stage of the disease. The overall five-year survival rate for cervical carcinoma in pregnant and unpregnant women is almost the same and the difference is not statistically significant (87.5% of women diagnosed with Stage Ib and 69.9% of women with Stage IIa survive their disease at least five years) [7]. The impact of pregnancy on the progression of cervical cancer is still disputable. It is believed that inhibition of cell-mediated immunity and high level of estrogens may have unfavourable impact on cancer growth. However, the majority of researches indicate that, as far as dysplasia, preinvasive and microinvasive cancer is concerned, the course of the disease among pregnant women is slow and pregnancy does not influence prognosis and overall survival rate in invasive cancer [10]. The treatment depends on the stage of the disease, weeks of gestation, general condition of the pregnant woman, and her attitude towards termination of pregnancy. It should be also conducted interdisciplinary and individually selected [4, 9, 11, 12].

Case Report

A 35-year-old multipara at 36 weeks of gestation in her third pregnancy was admitted to I Department of Obstetrics and Gynaecology of the Medical Centre of Postgraduate Education in Warsaw on 11th of May 2009 in order to finish the pregnancy followed by Wertheim-Meigs radical hysterectomy.

The patient at 27 weeks of gestation was previously admitted on 9th of March 2009 to the Department of Pathology of Pregnancy Municipal Hospital due to gestational diabetes mellitus (GDM G1). In her past obstetric history, she had two pregnancies which were physiological and finished at term. During the stay she was informed about diabetes diet and the rules of glycemic control. In consequence of the fact that she did not have a recent cytology result (last Pap test 2007 – the second group) and abnormalities on speculum examination, she was additionally recommended to have cervical cytology carried out. The patient was discharged from the hospital with saved and live pregnancy. On 20th of March 2009 the cervical cytology was performed. The test revealed high grade squamous intraepithelial lesion (HGSIL) according to the Bethesda System 2001 and a colposcopy was ordered. It was carried out on 14th of April 2009 and confirmed the result of Pap test. The result of the colposcopy biopsy from 15th of April 2009 was carcinoma palnoepitheliale akeratodes infiltrans colli uteri.

Before surgery she was informed about the disease and treatment possibilities and she consented to proposed treatment. On admission, speculum examination revealed as follows: oversized ectocervix, cervical ectropion which was partly covered with normal epithelium, ulceration four cm in diameter limited to the anterior lip, and purulent excretion from endocervical canal. USG examination revealed longitudinal lie, vertex presentation, mean gestational age 37 weeks, estimated fetal weight 3,150 grams, anterior placenta, and normal amount of amniotic fluid.

MRI of lesser pelvis performed on 14th of May 2009 revealed rotated and off-center cervix, diameter: four cm and unclear stroma. In the left lateral side of the cervix and in the anterior lip there were two focuses with a high signal on T2-weight images which sizes were as follows: 7×5×26×9 mm. If all clinical findings were in accordance, it could be invasive malignancy limited to the cervix. There were no signs of invasion into parametrium and vagina. The ovaries were moved by oversized uterus beyond the examined area. Obliteration of the nearest adipose tissue was probably the result of edema and impaired venous circulation due to pregnancy. The lymph nodes enlargement in this area was not observed.

The patient was qualified to cesarean section, hysterectomy with ovaries (on her request), and pelvic lymphadenectomy. On 15th of May 2009 cesarean section was performed using the suprapubic transverse incision. Female infant was delivered 3,520/54, Apgar 10. Afterwards there was performed the Wertheim-Meigs total hysterectomy with ovaries and upper part of the vagina and pelvic lymphadenectomy. A drain in the peritoneal cavity and in the obturator cavity was left. Estimated blood loss: 800–1,000 ml. The perioperative course was uncomplicated and there was no need of transfusion. On 20th of May 2009 the patient and her baby were discharged from the hospital in a state of good health.

Histopathological examination obtained on 27th of May 2009 revealed that macroscopically the cervix was 6.5 cm length and five cm in diameter. An ulceration 4.5 cm in diameter and 0.2 cm in depth could be observed within whole surface of the cervix. In the scope of the posterior lip of the cervix and the external os, there was a white tissue of tumor which constituted up to 90% of the cervical wall and stretched 2.5 cm into cervical canal. The vaginal cuff was 2.5 cm in width without invasion. The dimensions of uterine corpus were 14×13×11 cm, and there were no pathological changes. The fallopian tubes and ovaries were with no pathological changes. Microscopically: carcinoma planoepitheliale microcellulare precipuae akeratodes colli uteri. There was observed an invasion of the tumor into the cervix but not into the uterine isthmus. Right parametrium was almost without pathological changes. The left one: in the scope of one vessel there were emboli consisted of carcinoma cells. The vaginal cuff had normal appearance. The common iliac lymph node on the right side: in three nodes subcapular metastases were observed. The obturator lymph nodes on the left side: in three nodes there was an inflammation. The internal iliac lymph nodes on the right side were without significant changes. The internal iliac lymph nodes on the left side: in one lymph node there could be observed metastasis which filled up to 50% of its volume. There were no pathological changes in the left common iliac lymph nodes, the right obturator lymph nodes, and the right internal iliac lymph nodes.

The patient was referred to the Oncological Centre in Warsaw due to adjuvant therapy. She underwent radiotherapy of the pelvis. The dose of radiation was 4,500 cGy/g/d.fr.180 cGy/g and additionally there was a boost on the left external iliac lymph nodes performed in a dose of 1,500 cGy/g/d.fr.60 cGy/g. The treatment course lasted 33 days. During radiotherapy the patient received five courses of chemotherapy – DDP – 65 mg. From 5th to 19th of October 2009 she underwent brachytherapy HDR located in the top of the vagina in a dose of 22.5 Gy in three fractions up to 0.5 cm depth. The treatment tolerance was good.

In January 2010 CT examination of the abdomen and true pelvis revealed a solid tumor in the abdominal wall with dimensions of 40×60×43 mm. It was situated in the right rectus abdominal muscle on the level of the iliac crest. It was probably a metastasis. The patient was hospitalised from 7th to 12th of February 2010 in I Department of Obstetrics and Gynaecology of the Medical Centre of Postgraduate Education in Warsaw where she underwent a resection of the tumor. Histopathological examination confirmed the presence of carcinoma planoepitheliale's metastases. The pa-

tient was again admitted to the Department because of recurrence of the disease with vomit and severe pain in the area of pubis symphysis. Physical examination on admission revealed a disruption of wound in low pole and presence of purulent excretion was obtained which revealed numerous colonies of Escherichia coli. Sonography showed heterogenous area (sizes 25×20 mm) stretched to the abdominal wall. The abdominal X-rays performed in a sitting position revealed numerous fluid levels in a small and large intestine which indicated obstruction. Surgeon was asked for consultation due to suspicion of a fistula of large intestine and the symptoms of obstruction. The patient was qualified to parental nutrition. Rectal culture was also ordered. It revealed a large growth of Escherichia coli and Enterococcus faecalis. She was prescribed cefuroxime 3×1.5 g iv., metronidazole 3×500 mg iv., diclofenac 100 m 2×1 susp per rectum, nadroparin 0.3 ml sc, and morphine as analgesic.

PET–CT examination (March 24, 2010) which was performed out of hospital showed three small tumors up to 5.5 mm in the right lung, however no accelerated metabolic activity was observed. Apart from that there were no changes in the lungs. The thoracic lymph nodes were not enlarged. A large lesion was observed in the abdominal wall from S2 level to pubic symphysis (71.3 mm). Its largest transverse size (at the level of the top of hip joint) was 62.3×43.5 mm. The lesion revealed accelerated metabolic activity, SUV max 14.5. It did not invade the urinary bladder. There were single implants shown in the peritoneum. The lymph nodes were not enlarged. There were no focal lesions observed in the liver, spleen, and in the kidneys. Probably, as a result of inflammation, the accelerated metabolic activity in the stomach's and duodenum's walls was observed. CT examination (April 7, 2010) revealed an infiltration into peritoneum, omentum, and abdominal wall below a postoperative scar. In the lesion area was observed widened intestinal loop over this lesion with symptoms of subobstruction. The lesion stretched to the urinary bladder which front wall was altered and thickened.

Laboratory tests were collected according to the special procedure for patient suffering from cancer. Morphology (April 15, 2001): leukocytes – 10.2 K/ul, erythrocytes – 3.02 M/uL, hemoglobin – 8.31 g/dl. Other blood tests: urea – 22 mg/dl, creatinine – 0.8 mg/dl, protein – 6.6 G/DL, glucose – 119 mg/dl, albumin 3.2 g/dl, iron 21 ug/dl, CRP – 193 mg/l, ALAT – 22 IU/l, ASPAT – 15 IU/l, and LDH – 51 IU/l.

Because of worsening patient's state of health, the fistula of large intestine and the mechanical obstruction as the result of cancer recurrence, the patient was referred for the surgery. On 22nd of April 2010 she underwent partial resection of small intestine, colon, and resection of malignancy situated in the true pelvis and abdominal wall which was not radical. The ileum was repaired end-to-end and the transverse colon was repaired end-to-side. A proximal colonostomy, appendicectomy, and drainage of the small intestine were performed . During perioperative period two units of PRBCs and two units of FFP were transfused.

The results of laboratory test which was performed after surgery: morphology (April 22, 2010): leukocytes – 16.7 K/ul, erythrocytes – 3.86 M/uL, and hemoglobin – 10.8 g/dl. Other blood tests: urea – 18 mg/dl, creatinine – 0.5 mg/dl, protein – 5.3 g/dL, and albumin – 2.1 g/dl.

The patient was discharged from the hospital and was referred to the Oncological Center in Warsaw due to adjuvant radiotherapy and chemotherapy, however she did notcontinue the therapy. A few months later she was admitted to the I Department of Obstetrics and Gynaecology of the Medical Centre of Postgraduate Education again due to the worsening of her state of health and enterocutaneous fistula. This delay of the proper treatment may have been responsible for this unfavourable course of disease. She was referred to alternative continuance of the chemotherapy in the Oncological Center in Warsaw but she was disqualified from chemotherapy trials because of the worsening state of health and the stage of the cancer. Patient died in September 2010.

Discussion

Extended radical hysterectomy is a treatment of choice in case of Stage Ib cervical carcinoma associated with pregnancy. The aim of this treatment is the total resection of cancer and also the assessment of disease stage. The collected material allows to conduct histopathological examination. The result of this examination is important to make the decision concerning the relevant treatment – radiotherapy or chemotherapy. The extended radical Wertheim-Meigs hysterectomy is an extensive surgery and it is associated with a high risk of complications. The complications' frequency oscillates from several to 70% [9]. The complications are usually as follows: damage of the nervous system structures and ligaments that maintain the normal position of the uterus, damage of parametrium, vesical, and perirectal venous plexuses, as well as venous plexus of the obturator cavity, damage of the ureter, urinary bladder and large arteries and veins during resection of the pelvic, and para-aortic lymph nodes [9]. The frequency of these intraoperative complications is estimated from 0% to 16% [13]. The intraoperative mortality is assessed from 0% to 2% [8]. The frequency of postoperative complications occurring among patients who have undergone Wertheim-Meigs hysterectomy is quite common [9]. The peculiarity of Wertheim-Meigs hysterectomy and its range cause higher and additional risk of complication among pregnant women who are treated because of cervical cancer. At the same time, adjuvant therapy may also caused some complication, for example the intestinal injury which concerned in the present patient. These can be divided into early and late radiation complications. Early severe complications can be observed in 1.1% of patients whereas early moderate radiation complications occur in 41% of patients. Moreover they are more frequent among patient treated with surgery and adjuvant radiotherapy than in patients treated with radiotherapy alone. The second risk factor is the time between surgery and radiotherapy, especially when it is less than four weeks [14]. The pregnancy is a state which constitutes additional risk of complications [7]. The lesser pelvis of pregnant women is highly vascularised and the performing of such extensive surgery followed by cesarean section is associated with even higher risk for patient than mentioned above. The high level of perimetrium's and perivaginal's vascularisation among pregnant women is connected with higher risk of intraoperative and postoperative complications in connection with unpregnant women. During preparation for the surgery it is very important to perform MRI examination which allow to assess the stage of the disease. In spite of high risk of complications, extended radical hysterectomy is the only known surgery

which may save both mother's and child's life. The prognosis depends on the stage of the disease, patient's age, state of health, socio-economic status, as well as the level of medical knowledge and technical opportunities of the hospital [2, 4, 8, 15]. Identification of the full list of predictor factors, doctors' appropriate knowledge, and experience allow choosing the best treatment procedure. The decision should always be taken after consultation with the patient. She must be informed about advantages and disadvantages of the proposed treatment and possible consequences for the infant because of preterm delivery. During pregnancy, woman is more often examined than ever before. The proper diagnosis before pregnancy is a very important prognostic factor. The prognosis is better if the diagnosis is known at early pregnancy because of the possibility for immediate treatment. The diagnosis in advanced pregnancy is usually associated with higher stage of the disease and higher risk of recurrence and complication which may lead to patient's death [15].

According to Polish Gynecological Society Guidelines, all women should begin cervical cancer screening three years after they begin having vaginal intercourse, but no later than when they are 25-years-old. Screening should be conducted every year with a regular Pap test. Women who have normal Pap test results and do not have any additional risk factors of cervical cancer may get screened every three years. Women older than 30 who have had three normal Pap test results in a row or women after total hysterectomy may also get screened every three years. Every year examination is required for women who have HIV infection, oncogenic HPV types infection, and those who are taking immunosuppressive drugs [16]. Moreover Polish Gynecological Society recommends performing Pap test before conception if the last Pap test has been performed over six months earlier and/or during first antenatal appointment with a gynecologist [17]. These guidelines had been followed by the present team, but it did not protect the patient before the development of invasive cancer. In different countries, cervical cancer screening programs are similar to this performed in Poland. Usually, it is recommended to begin cervical cancer screening at 20-30 years and extending to 60-65 years, at a three- or five-year intervals [18]. The duration of progression from a precancerous phase to cervical cancer is quite long, and it is estimated to occur at ten to 12 years [19]. The progression of disease in the present authors' patients is surprisingly fast and it may dispute their current knowledge regarding tumor biology during pregnancy, especially in the third trimester. Unfortunately, the management guidelines for these patients remain unclear. Some authors have recommended that maximum delay of treatment may be 12 weeks for Stage Ib1 tumors and six weeks for Stage Ib2 tumors [20]. According to French recommendations for management of pregnant patients with invasive cancer, in case of Stage Ib2 cancer diagnosed after 22 weeks of gestation, the acceptable delay in treatment should be less then six to eight weeks [21]. However, in other studies the delay of definitive treatment of Stage Ib of disease only for four and six weeks led to patients' death [22].

The present patient was admitted to the hospital because of gestational diabetes mellitus. There were abnormalities observed on speculum examination. It is controversial why colposcopy or at least a cervical cytology during her first hospitalization was not carried out. These tests could accelerate the diagnosis of cancer. In this case the time between the first hospitalization and the histopathological examination's result was about 40 days. The result of her previous Pap test was the second group. Usually the progression to cancer is slower than two years. The reason of this unexpected fast progression might be false negative result of cervical cytology. It should be remembered that the sensitivity of cervical cytology is only about 70-80%. The second reason of this situation might be the fact that pregnancy is a state which helps the development of cervical cancer via two mechanisms. First is the fact that higher vascularisation of lesser pelvis is probably responsible for faster progression of the disease and metastases. Moreover changes in the activity of immune system may weaken the immune response. It may be considered whether the pregnancy should be terminated after 34 weeks of gestation after the previous steroid therapy. Many researchers claim that after this time infant's life expectancy is similar to that which occurs among full-term infants [21, 23-26]. It should be also obligatory to perform a Pap test in the first trimester of pregnancy regardless of last cytology result. If there are any doubts, a colposcopy should be performed.

As long as we do not have clear recommendations about the management of cervical cancer during pregnancy, this tumor will still remain a clinical challenge. Apart from this, treatment of these women in specialistic hospitals and hastening of diagnostic procedures, particularly during third trimester, seems to be reasonable. Verification of screening scheme during pregnancy should be also considered, especially in a group with additional risk of cancer development.

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A large breast lump causing a diagnostic dilemma

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Summary

Lipomas are the most frequent mesenchymal tumors consisting of mature fat cells and are usually benign. They represent approximately 4-5% of all benign tumors that occur in human body. They can sometimes present very large sizes in their localization and are referred to as "giant lipomas". In this article, the authors report an unusual case of a right breast giant lipoma causing diagnostic dilemma. A 62-year-old woman was referred to the present hospital with a complaint of a sudden chest asymmetry of the right breast increasing at the connection of the pectoralis muscle. Ultrasonography revealed breast tissue involution (ACR 1). Specifically, the ultrasound findings were mostly compatible with lipid mass (lipoma) and areas with cystic necrosis. The findings from digital mammogram were not conclusive compared with ultrasound examination. Moreover, the results from the breast MRI were contradictory and other diagnosis was evinced. The patient underwent wide-surgical excision and reconstruction and had an excellent postoperative issue. According to the final histopathological examination, the tumor measured 17 cm and was covered by a thin membranous capsule. Furthermore, it had the appearance and composition of adipose tissue. In conclusion, according to the authors' view, this case is rare due to its challenging size and the difficulty in differential diagnosis.

Key words: Breast lipoma; Giant lipoma; Rare breast lump; Benign breast tumor.

Introduction

Lipomas are ordinarily benign tumors [1, 2]. In vast majority, lipomas are small and usually do not enlarge expeditiously [3]. Moreover, in most cases, their appearance is sporadic, with no known cause. Although lipomas may occur in any part of the body and are composed mainly of fat tissue [4], they rarely develop in the breast causing diagnostic dilemma [5]. It is well known that mammography and ultrasonography are often the two basic imaging tools in case of breast diseases [6] and by extension when a palpable mass is found. However, often these tools are not capable to distinguish a lipoma from other conditions. The unusual case of a right breast giant lipoma prompted the idea of writing this report mainly because of its extremely challenging size and due to the difficulty in differential diagnosis with the use of the available imaging techniques.

Case Report

A 62-year-old female came to REA hospital in Athens with a complaint of chest asymmetry of the right breast increasing at the connection of the pectoralis muscle. This symptom appeared a few two to three months prior. Clinical examination showed a palpable, movable, and painless mass in the right breast, without the presence of nipple discharge or axillary lymphadenopathy. The laboratory dosage of CEA and CA 15-3 were normal.

Ultrasonography revealed breast tissue involution (ACR 1). Moreover, a radiolucent mass (lipid tissue), two cm in diameter, close to major thoracic muscle was observed, and the muscle appeared thinner. Furthermore, the ultrasound showed that in the center of the mass there were three lipid cysts in contact with a diameter of four cm. In the additional work-up with shear wave elastography (SWE), the area presented a low mean value (17.0 kPa) and SWE-Ratio (1.23). Consequently, the ultrasound findings were mostly compatible with lipid mass (lipoma) or liposarcoma and areas with cystic necrosis.

The findings from digital mammography were classified into the BIRADS-2 category. It is noteworthy that mammography detected a scattered fibroglandular tissue, especially in upper outer quadrant of the breast. Additionally, mammography confirmed all the aforementioned findings from the ultrasound examination. Compared with the previous mammogram, there was no significant change in the radiographic appearance, except from the lesion in the right pectoralis major muscle.

Breast magnetic resonance imaging (MRI) exam was performed at axial, sagittal, and coronal planes, before and after the intravenous gadolinium contrast enhancement. According to the findings, there was a lesion consisting mainly of fat tissue under the right major pectoralis muscle. In the center of this lesion, a secondary multilobulated lesion approximately four cm in diameter, was recognized. This secondary lesion appeared with high intensity T1 signal and medium-to-low signal on T2-weighted images, while intravenous contrast injection showed linear peripheral enhancement. The differential diagnosis of this image was between accessory breast, lipomatosis or lipoma under the major pectoralis muscle, containing an area of fat necrosis. Neverthe-

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Figure 1. — Drawing of the surgical scars.

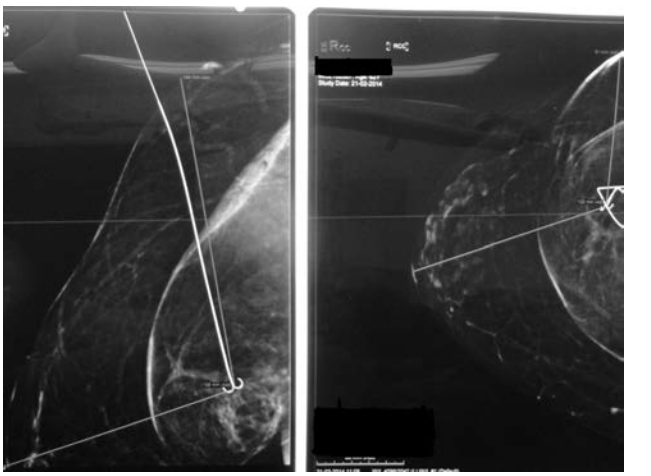

Figure 2. — Mammographic view (MLO and CC) with the hook wire.

Figure 3. — Wide-surgical excision and incision of the subcutaneous fat.

Figure 4. — Incision of the pectoralis muscle.

Figure 5. — Removal of lipoma.

Figure 6. — Giant breast lipoma.

Figure 7. — Lipoma measuring 17 cm.

Figure 8. — Excised specimen.

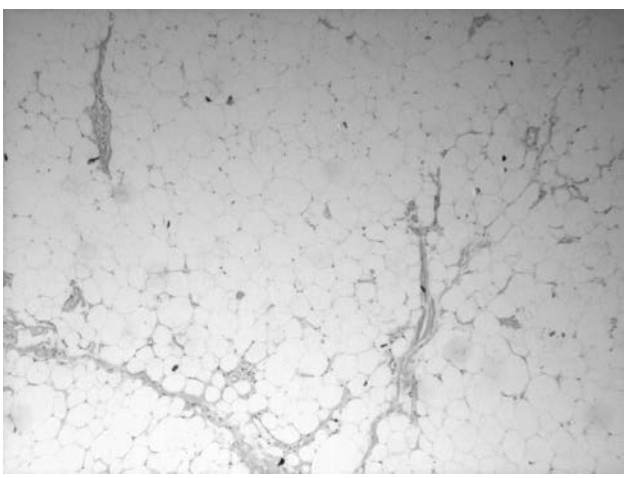

Figure 9. — Breast lipoma H&E ×25.

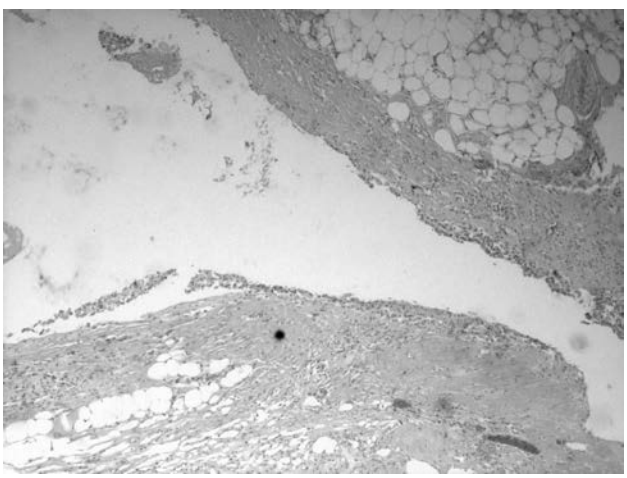

Figure 10. — Area of connective tissue lined by foamy histiocytes ×25.

less, the last case was the most probable one. There was no area of abnormal enhancement that indicated invasive lesion of the breast after the intravenous gadolinium contrast injection. Moreover, there was no axillary adenopathy bilaterally.

Despite the probable benign condition, the patient was advised to have it removed by surgical procedure. The first step of the surgery was the exact location of the breast abnormality with the usage of a fine hook wire preoperatively (Figures 1, 2). The patient underwent wide-surgical excision under general anesthesia (Figures 3-5). An arcuate incision of lump was performed in the upper external quadrant of the right breast along the Langer's lines. After opening the subcutaneous tissue, the authors discovered that the major pectoralis muscle was very thin and dislocated. They opened with electrical scalpel the muscle longitudinally and the lump appeared and was grasped out from the scar. A precise hemostasis was performed and reconstruction of the defect was made with local gland flaps (Takeda technique). The surgical cavity was drained with a negative pressure aspiration (wound vac). The result from the frozen section was negative for malignancy and specifically the specimen was diagnosed as lipoma and liponecrosis (Figures 6-8). According to the final histopathological examination, the tumor measured 17×14×8 cm with a total weight of 1,789 grams and covered by a thin membranous capsule (Figures 9-12). Furthermore, it had the appearance and composition of adipose tissue. The patient had an excellent postoperative performance status and was discharged the same day.

Discussion

Lipomas are the most frequent mesenchymal tumors consisting of mature fat cells and are usually benign, well- circumscribed, and encapsulated [5, 7]. The incidence of lipomas ranges from approximately 10% to 16% in case of mesenchymal tumors [1, 8-10] and also, rep-

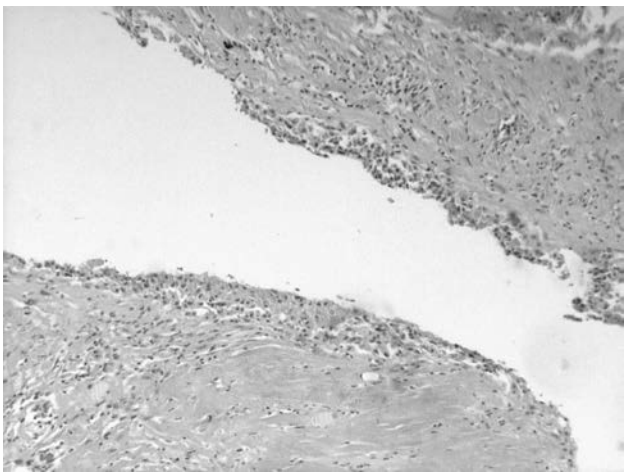

Figure 11. — Chronic inflammatory infiltrate and foamy histiocytes (H&E ×50).

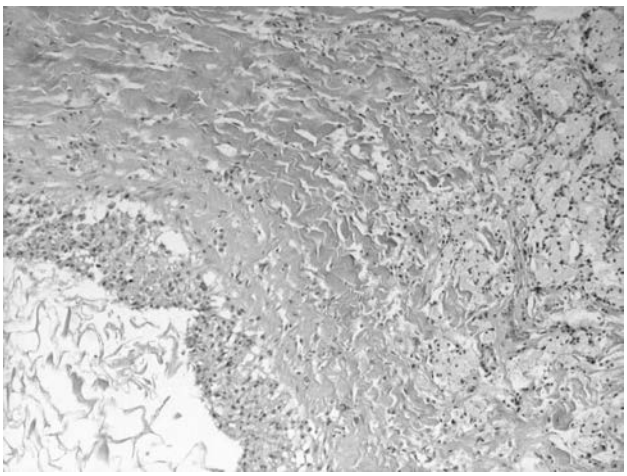

Figure 12. — Area of liponecrosis (H&E ×25).

resent 4-5% of all benign tumors that occur in human body [11]. Owing to the limited information in the literature concerning lipoma occurring in breast tissue, the exact incidence of breast lipoma remains a subject of intense controversy [11]. Moreover, the etiology of lipoma development is not completely understood [4, 12]. It is worthwhile to note that lipoma may affect all people independent of their sex and age. As an example, there are several case reports that describe breast lipoma development in men [10]. Moreover, at times the patients may have more than one lipoma in their body and this is due to a genetic condition, which is known as familial multiple lipomatosis [4]. Lipomas are also known to develop in children, as shown by a study which conducted by Greek scientists [13], but its occurrence in this population is rare.

Giant breast lipomas are defined as tumors that have a diameter of at least ten cm in one dimension or weight of more than 1,000 grams [1,9-11]. Giant breast lipomas have rarely been reported in the literature and for this reason the present case report is rare due to the challenging tumor size. Owing to the normal fatty composition of the breast, breast lipoma may cause diagnostic uncertainty [5, 14]. Thus, difficulties in differentiation from other breast lesions are often encountered [1, 8]. The differential diagnosis of lipoma includes benign conditions: hamartoma, hematoma, haemangioma, angiomyolipoma, cyst, abscess, fat necrosis, fibroadenoma, accessory breast tissue, and malignant breast conditions: liposarcoma and carcinomas, also miscall breast conditions: as phyllodes tumor [5, 6, 9, 15, 16].

The data of the literature are in accordance that there is almost no risk of subsequent malignant transformation associated with lipomas [1, 2, 4]. However, if lipomas are > ten cm they could contain sarcomas tissue and for this reason biopsy is required [12].

Undoubtedly, breast ultrasound and mammography are the two basic tools for the differential diagnosis of breast lumps [6, 9]. MRI can sometimes assist in difficult situations. However, the final diagnosis is made by core or "open excisional biopsy" [2]. It is noteworthy that in case of a clinical diagnosis of lipoma, a biopsy is required if the suspected lipoma causes symptoms as pain, movement restriction, and rapid enlargement or soft consistence [4].

The standard and definitive treatment of a lipoma should be surgical excision [12, 17, 18]. It is noteworthy, that some published scientific studies indicated that in some cases the endoscopic-assisted suction of lipomas with the usage of an ultrasonic scalpel may offer a better and a less invasive surgical result [19, 20]. After surgery, the most common complications are seroma, hematoma, infection, and scars [4]. Also, other modalities of treatment can be proposed: liposuction is an effective alternative treatment of giant lipomas as shown by two studies [5, 21] and has been associated with good cosmetic results [11]. Despite the fact that Raemdonck *et al.* demonstrated that there is a high percentage of recurrence after liposuction in comparison to surgical excision [22], a more recent study by Al-basti *et al.* reported that there was no sign of recurrence after liposuction in a six-year postoperative follow up [23]. However, this technique needs to be improved due to its side effects [11]. Another alternative treatment of lipomas is "chemical lysis" by subcutaneous injection of deoxycholate which creates lysis of the adipose tissue [24]. However, further studies are essential before this treatment can be routinely recommended [4].

Although successful excision leads to an excellent prognosis [1], follow-up is necessary due to the possibility of relapse after a few years [11]. In conclusion, despite the benign behavior of lipoma, because of the symptomatology, it is necessary to remove it in order to obtain final histopathological results.

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EJGO European Journal of Gynaecological Oncology

A case report of benign metastasizing leiomyoma of the lung: FDG-PET-CT findings and the utility of uterine needle biopsy

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Summary

Benign metastasizing leiomyoma (BML) is a rare condition that affects other organs out of the uterus. Recently, a few case reports in which 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) has been used to distinguish the malignancy have been published. Here, the authors present a case of BML with metabolic activity on PET, in which needle biopsy of the uterus was efficient to make diagnosis.

Key words: Benign metastasizing leiomyoma; FDG-PET-CT; Needle biopsy.

Introduction

Benign metastasizing leiomyoma (BML) is an obscure condition in which metastatic benign smooth muscle lesions in the lungs, lymph nodes, or abdomen appear to be derived from leiomyoma of the uterus. Because of its rarity and uncertainty, making proper differential diagnosis from malignant tumors is still challenging. Here, the authors present a rare case of BML of the lungs with metabolic activity on 18F-fluorodeoxyglucose positron emission tomography (FDG-PET), in which needle biopsy of the uterus was efficient to make a decision on further treatment.

Case Report

A 51-year-old premenopausal asymptomatic woman was referred to the present hospital due to abnormal shadows on a chest X-ray. She had a history of myomectomy at age 35. Chest computed tomography (CT) revealed more than 30 multiple nodules in both lungs (Figure 1), and metastatic lung cancer was highly suspected. PET-CT with FDG was performed to identify the primary carcinoma site and find any other metastatic lesions. PET-CT showed high FDG uptake on the uterus. The maximum standardized uptake value (SUVmax) on the uterus was 24.7, whereas on the lung lesions it was 2.5 (Figure 2). On magnetic resonance imaging (MRI) of the pelvis, an enlarged uterus and multiple round nodules were found (Figure 3). With these imaging investigations, the authors considered uterine sarcoma as the origin of the lung metastasis.

The treatment strategy for advanced uterine sarcoma is still controversial. The present authors' policy for uterine sarcoma with unresectable metastasis is combined chemotherapy. To make pathological confirmation, therefore, bronchoscopic biopsy of lung nodules was performed. This revealed estrogen receptor-positive spindle cells; however, it was not possible to confirm BML or sarcoma. The authors then performed trans-abdominal needle biopsy of the uterus. This showed spindle cells as well and was compatible with leiomyoma. Finally, the diagnosis of BML of the lungs was made. With this diagnosis of BML, total hysterectomy and bilateral salpingo-oophorectomy were performed.

Discussion

Here, the present authors found two important clinical issues. BML of the lungs can show metabolic activity on FDG-PET-CT, and uterine needle biopsy is useful for the proper diagnosis of this condition. First, BML of the lungs can show metabolic activity on FDG-PET-CT. Nowadays, FDG-PET-CT has become an accepted tool and plays an important role to distinguish malignant from benign tumors. For the evaluation of pulmonary malignancy, the sensitivity of FDP-PET-CT is about 90% with a specificity of around 78% [1]. With regards to SUVmax in the pulmonary nodules, SUVmax greater than 2.5 is suspicious for malignancy in the appropriate clinical settings [2, 3].

To the best of the present authors' knowledge, only four cases of BML with FDG-PET-CT findings have been reported in the English literature [4-6]. The SUVmax was from negative to 2.2 in those prior cases. A case with SUVmax 2.2 had open thoracotomy carried out for the pathological confirmation [5]. Compared to these four cases, the current case showed relatively high FDG avidity in the lung nodules. Furthermore, the excessively high SUVmax in the uterus made it difficult to exclude that the metastatic lung cancer originated from the uterine sarcoma. In addition, it is well known that leiomyoma of the uterus show high SUVmax on FDG-PET-CT [7-9]. Nishizawa *et*

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Figure 1. — Chest CT showing multiple round nodules of various sizes in both lungs.

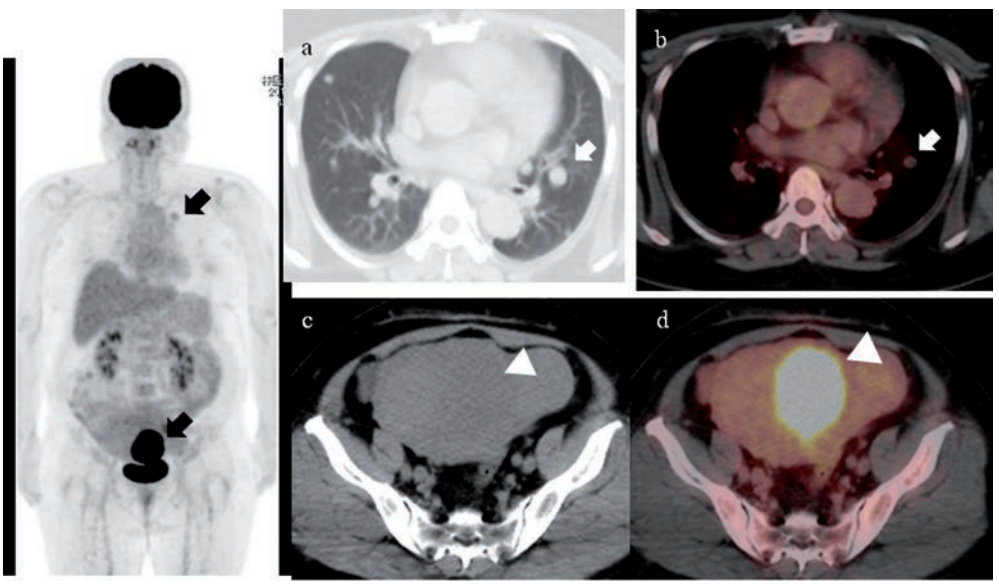

Figure 2. — FDG uptake on PET-CT. SUVmax on the lung lesions is 2.5 (a, b), whereas on the uterus it is 24.7 (c, d).

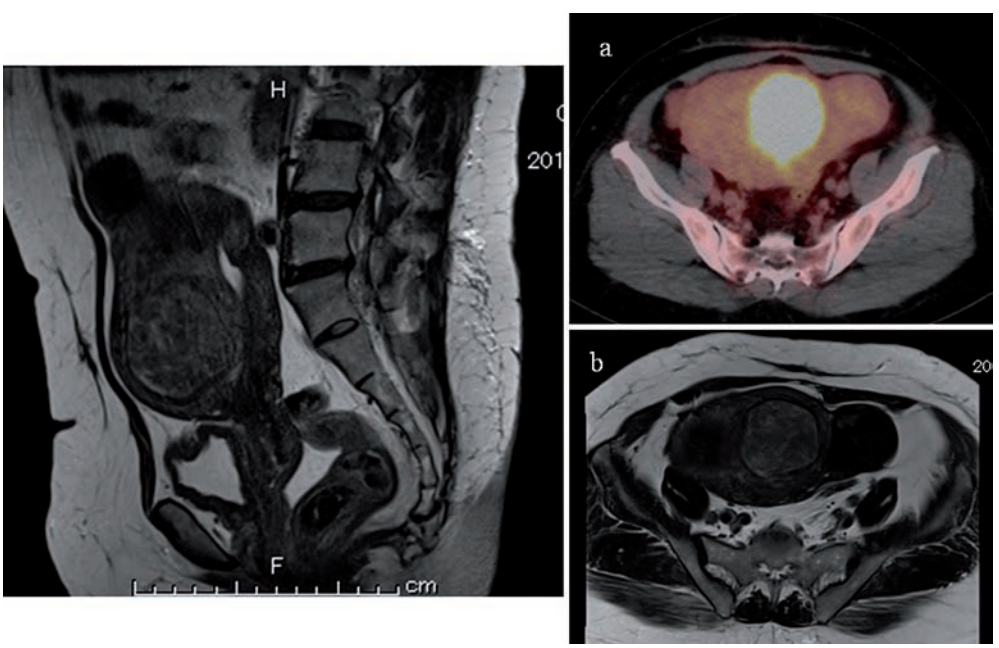

Figure 3. — T2-weighted MRI revealing an enlarged uterus and multiple round nodules. Comparison of a nodule by PET-CT (a) with MRI (b).

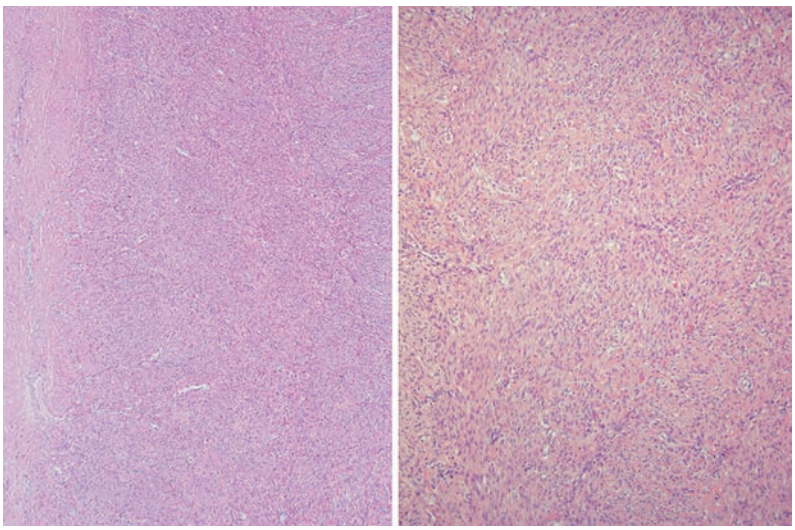

Figure 4. — A surgically removed uterine tumor had moderately greater cellularity for leiomyoma, although mitosis is sparse and coagulative tumor cell necrosis is absent.

al. reported a FDG-PET-CT screening in 1,357 healthy women, and found an SUVmax larger than 3.0 in 10% of the leiomyomas in premenopausal women, and 1.2% in postmenopausal women[10].

Another prospective study showed 17% of leiomyomas had SUVmax higher than 2.5; however, only 1.6% had one larger than 5.0 [11] The reason for high FDG uptake in leiomyomas is suggested to be related to the high levels of glycogen in a myomatous uterus, the increased blood fraction, and the proliferation of the smooth muscle cells due to increased metabolic need [7]. The SUVmax in sarcomas is supposed to be significantly higher than in leiomyomas, but its limits to differentiate these two neoplasms are still uncertain [12]. In the present case, SUVmax on the uterus was extremely high, as in benign leiomyoma, up to 24.7. Microscopically, surgically removed uterine tumors had moderately greater cellularity than the surrounding myometrium (Figure 4). Retrospectively, the present authors conclude that these pathological findings could account for the high SUVmax. Second, uterine needle biopsy is useful for the proper diagnosis of this condition. With only imaging evaluation, preoperative distinction of uterine sarcoma from leiomyoma is sometimes difficult. Especially because the current case was suspected for malignancy with metastatic lung cancer, pathological confirmation was essential to make the clinical decision on further treatment. Needle biopsies are frequently performed on solid tumors in other organs such as the prostate, breasts or liver, however, its utility for uterine tumors has not achieved widespread acceptance. Two prospective studies of uterine needle biopsy have been published so far [13, 14]. Kawamura *et al.* analyzed 435 patients with 'myoma-like' nodules by MRI, in which seven had uterine sarcoma. Combined histopathologic findings of transcervical needle biopsy and MRI, gave sensitivity and specificity with respect to distinguishing uterine sarcoma from leiomyoma of 100% and 98.6%, respectively [13]. Tamura *et al.* described that in 63 cases of uterine tumors determined by MRI, 12 were diagnosed as malignant by needle biopsy, and 51 cases were benign. Among these 51 patients, one was diagnosed as having a low-grade endometrial stromal sarcoma post-surgically. They found the sensitivity and specificity of the needle biopsy were 91.7% and 100%, respectively, and concluded this may be a reliable preoperative diagnostic procedure for uterine tumors with suspicious malignancy [14].

The present authors performed ultrasound-assisted transabdominal needle biopsy of a uterine tumor that had high FDG avidity. It revealed spindle cells without atypia and absence of coagulative tumor cell necrosis (Figure 4). With these pathological findings, the diagnosis of BML of the lungs was finally made.

In conclusion, BML of the lung can show metabolic activity on FDG-PET-CT, and uterine needle biopsy is useful for the proper diagnosis of this condition. Further reports should be accumulated to determine whether BML with FDG avidity may be more common than previously thought, and whether needle biopsy may be useful to make proper decisions in such cases.

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Endometrial polyp harboring a primary B-cell non-Hodgkin lymphoma: a useful in-office hysteroscopic approach

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Summary

A non-menopausal women underwent gynecological evaluation for spotting and menstrual irregularities. After first line gynecological assessments, the patient underwent office hysteroscopy. By using an in-office technique, two isthmic endometrial polyps and one cervical polyp were removed. One endometrial polyp was found to harbor a B-cell high-grade lymphoma just on the apex (found to be necrotic). The following staging examinations and the pathological assessment of the endometrium, the uterus, the adnexa, and the lymphatic regional nodes did not find any localization of the lymphoma. No additional treatments were done. The patient is alive and disease-free at 18 months follow-up.

Key words: Endometrial polyp; B-cell lymphoma; Hysteroscopy.

Introduction

Lymphomas of the genital tract are uncommon. Some cases reported in literature demonstrate the possibility of the primary onsetting of lymphomas in the genital tract, usually presenting as B-cell lymphomas and sometimes multifocal lymphomas [1]. In only four cases, a lymphoma was found in endometrial polyps to the best of the present authors' knowledge [2-5].

Herein, a case of a primary B-cell lymphoma arising in an endometrial polyp is reported in order to improve the knowledge about this exceptional situation.

Case Report

A non-menopausal women, aged 53 years, presented to her own gynecologist to check menstrual irregularities and spotting. Her personal history was uneventful. The gynecologist performed a gynecological examination, a specular examination, a pap smear, and an ultrasonographic transvaginal scan. No abnormalities were found at the gynecological examination, at the specular examination, and at pap smear. The ultrasonographic transvaginal scan resulted in aspecific endometrial irregularity. Therefore, the patient was counseled to plan a hysteroscopy, to better understand the bleeding site in endometrium. The hysteroscopy was made with a Bettocchi hysteroscope, allowing endometrial biopsies or in-office operative procedures. The hysteroscopist detected two endometrial polyps and a cervical polyp. The hysteroscopic endometrial pattern was proliferative and normal, and the tubal orifices were normal. The endometrial polyps were both at isthmic level of the endometrium on the posterior wall of the uterus. In one of them, a necrotic area on the apex was detected as the bleeding site. All the polyps, both cervical and endometrial, were removed with 2.5 French forceps of the Bettocchi hysteroscope, by tractioning them from the base. This technique avoids polyps' morcellation and improves pathological diagnosis [6].

Unexpectedly, the histological examination showed that the apex of the bleeding polyp was infiltrated by atypical lymphoid cells, with large nucleus, along with multiple nucleoli and frequent mitosis (Figure 1). The lesion was two mm across the largest diameter. The immunohistochemical analysis demonstrated that the neoplastic cells were positive for CD20 and Bcl-6, and negative for CD3, CD5, CD10, CD23, Bcl-2, CD30, and Alk-1. The Ki67 proliferative index was 95%. Based on these finding and after a careful review of the slides, the diagnosis of a B-cell high-grade lymphoma was done.

The patient was urgently referred to hematological evaluation. Thus, a total body positron emission tomography (PET), a total body computerized tomography with contrast, a colonoscopy, and bone marrow sampling were performed. All these examinations excluded lymphatous localizations. Therefore, a primary localization of the B-cell lymphoma in the endometrium was strongly suspected. A laparothomic hysterectomy with annessectomy and regional lymphadenectomy was performed for staging and treating the disease. The pathological examination resulted in a normal pattern of the uterus, ovaries, and nodes. Therefore, it was concluded that the primary localization of the B-cells lymphoma was in the endometrial polyp. The postoperative course of the patient was uneventful. No other therapies were scheduled. Patient is fine at 18 months follow-up.

Discussion

To date, four other cases of lymphomas in endometrial polyps were reported [2-5]. In the case of Rittenbach *et al.* [2], large atypical lymphoid cells with immunophenotypic pattern of B-cell lymphoma were found in bioptic fragments of an endometrial polyp protruding from cervical os. The pattern of B-cell lymphoma within the polyp was con-

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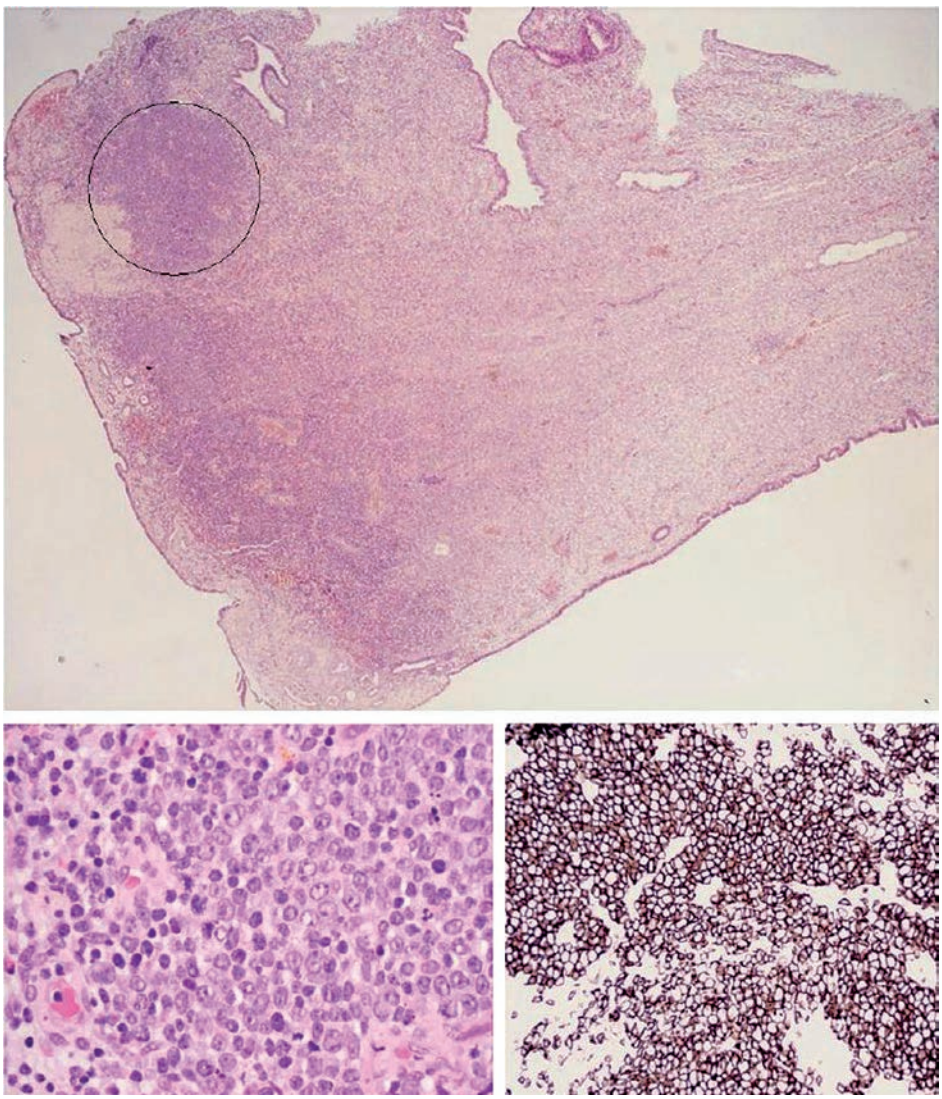

Figure 1. — Upper: the excised polyp contains an area of increased cellularity (circle, H&E, ×20). Bottom left: at higher magnification, the area is composed of blastic lymphoid cells (H&E, ×400). Bottom right: The neoplastic cells show strong and diffuse CD20 positivity (CD20, ×250).

firmed after total hysterectomy, because uterus, endometrium, and another endometrial polyp were not involved in the disease. Histological signs of chronic inflammation in the polyp did not involve malignancy, two leiomyomatas, and adenomyosis were also described. No antitumor therapy was done. After three years from surgery, patients was free from disease.

In the case of Annibali *et al.* [3], a B-cell MALT-type lymphoma was found after endometrial polyp removal. No other surgery and therapy was done and the patient was free from disease at 24 months follow-up.

More recently, Xia *et al.* [4] reported a case of a large intravascular B-cell lymphoma within an endometrial polyp. With just the removal of the polyp, the patient is alive at ten months follow-up. However, by reading the Xia *et al.* case [4], one can understand a diffusion of the disease at the time of endometrial polyp removal.

Additionally, Guldrís *et al.* [5] reported a primary diffuse non-Hodgkin B-cell lymphoma in an endometrial polyp and in endometrium. The disease was reported in two lymphatic nodes as well. A combined chemotherapy and radiotherapy were performed and remission was reported at 18 months follow-up.

The present case shares some similarities with the cases of Rittenbach *et al.* [2] and Annibali *et al.* [3]. In both cases, the neoplasm was confined to endometrial polyps, and seemed to have a favorable prognosis. However, in the present case, there was a large B-cell non Hodgkin lymphoma, like the one of Rittenbach *et al.* [2]. The Rittenbach *et al,* [2] case and the case presented here underwent both hysterectomy. The surgical removal of the uterus (and, in the present case, of the adnexa and of the lymphatic nodes) excluded the diffusion of the neoplasm to other sites of the genital tract. No additional therapies

were done, with good prognosis. Overall, endometrial primary lymphoma may onset like a polypoid lesion [1] or may associate to endometrial polyps [7]. Therefore, it should be recommended to quickly remove all the endometrial polyps and then the uterus in order to reach a diagnosis. It could be speculated that the endometrial polyp removal may be the first line surgical treatment for a localized primary B-cell non-Hodgkin lymphoma of the endometrial polyp. Moreover, tricks for improving the pathological assessment should be used. The in-office hysteroscopic procedure proves to be effective for both the pathological examination and a quick first line surgical therapy.

In conclusion, endometrial polyps may exceptionally harbor primitive B-cell non-Hodgkin lymphomas. The pathogenesis and the treatment of such exceptional situation needs further investigations, but a two-step surgical approach (polypectomy and hysterectomy) could be effective in the localized cases. The present authors recommend to report cases of lymphomas arising in endometrial polyps, due to the need of understanding the best treatment to schedule for such an exceptional condition.

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Ovarian clear cell carcinoma recurrence presenting as subcutaneous nodules

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Summary

Skin metastasis is a rare form of ovarian carcinoma spread and is associated with widespread disease and a poor prognosis. The authors present a case of a patient, with a past history of ovarian clear cell carcinoma, who presented with subcutaneous nodules as the first sign of recurrent metastatic disease.

Key words: Ovarian cancer; Ovarian clear cell carcinoma; Skin metastasis; Subcutaneous nodule; Case report.

Introduction

Ovarian cancer is associated with the highest mortality rate of all gynaecological malignancies in developed regions of the world and is the eighth most common type of cancer world-wide [1]. Epithelial ovarian cancers account for 90% of ovarian cancers. Ovarian clear cell carcinoma is a distinct subtype of epithelial ovarian cancer and often presents as a pelvic mass at FIGO Stage I or II disease [2-3]. Unfortunately ovarian clear cell carcinomas are associated with higher rates of recurrence and subsequent higher mortality rates compared with other epithelial ovarian cancers [4].

Epithelial ovarian cancers generally metastasise by direct seeding of the peritoneal cavity. Extra-peritoneal metastases occur in less than 40% of patients and in these cases, metastases to pleura, lung, and liver are well-recognised sites of distant ovarian cancer spread [5-6]. Subcutaneous nodules are a rarely encountered site of distant metastasis, occurring in about 3.5% of epithelial ovarian cancers [5]. The majority of subcutaneous metastases occur as a single nodule at the level of the umbilicus, known as the Sister Mary Joseph nodule, which is thought to occur secondary to direct peritoneal spread [5].

Subcutaneous metastases from solid tumours occur at a rate of 10%, most frequently from breast cancer and melanoma [7]. The diagnosis of subcutaneous metastases is commonly associated with extranodal metastatic disease and a poor prognosis [7]. In ovarian cancer, the median survival time from diagnosis of skin metastasis is nine months [7].

The authors present a case of a woman who presented with multiple anterior abdominal wall subcutaneous nodules confirmed to be metastatic ovarian clear cell carcinoma, and discuss the clinical implications of this finding.

Case Report

A 46-year-old female patient presented with a left ovarian mass, which was an incidental finding on a routine general examination. At the time of initial diagnosis, there was a large complex cystic mass arising from the left ovary on computerised tomography (CT). The Ca125 level was within the normal range at 20 U/ml. The patient underwent a total abdominal hysterectomy and left salpingo-oophorectomy, left pelvic lymph node dissection, and left para-aortic node sampling. She had previously had a right salpingo-oophorectomy. She was diagnosed with FIGO Stage 1C1 ovarian clear cell adenocarcinoma based on comprehensive staging. The left ovarian cystic mass was densely adherent to the pelvic peritoneum, pelvic side wall, uterus and rectum, and ruptured intraoperatively. Peritoneal washings were negative and there was no evidence of lymph node spread (0/11 lymph nodes positive). She was managed postoperatively with six cycles of chemotherapy with carboplatin and paclitaxel and no radiotherapy. A CT scan postoperatively demonstrated no evidence of distant metastases.

Four years after the initial diagnosis, the patient presented with intermittent abdominal pain and mild abdominal distension. A restaging CT demonstrated a left pelvic sidewall mass, which was confirmed to be ovarian clear cell carcinoma by fine needle biopsy. This was initially managed with three cycles of carboplatin and six weeks of radiotherapy. The carboplatin was ceased after the third cycle due to an anaphylactoid reaction. The following year a positron emission tomography (PET)-CT scan demonstrated that the left pelvic sidewall mass had increased in size. This was managed surgically, with a laparotomy, removal of an omental nodule, and division of adhesions. After a period of nine months, the patient presented with persistent vague abdominal symptoms, however a PET scan demonstrated no evidence of disease recurrence and the Ca125 level remained within the normal range.

At age 52, two months after this PET scan, and five years after the initial diagnosis, the patient noted a number of "lumps" in her abdominal skin and on examination there were multiple subcutaneous nodules in the anterior abdominal wall. A magnetic resonance imaging (MRI) scan demonstrated five new subcutaneous

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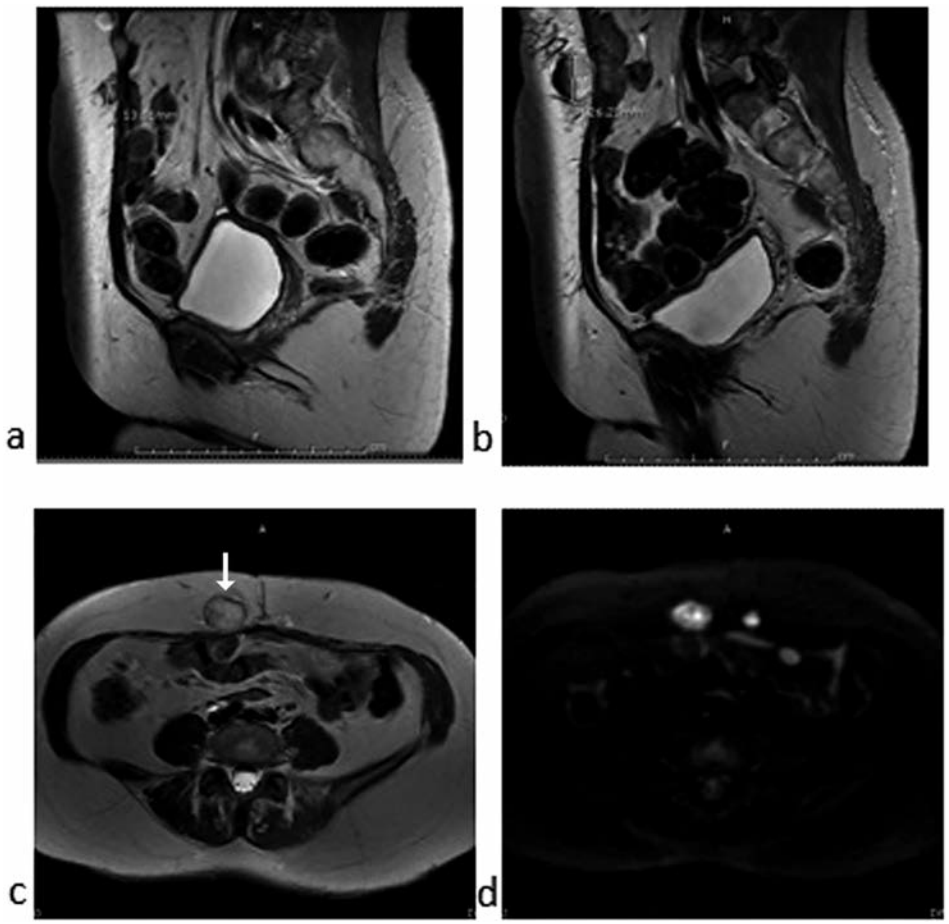

Figure 1. — a), b), c) MRI images demonstrating five new subcutaneous nodules in the anterior abdominal wall. The largest nodule is to the right of the midline scar below the umbilicus in the subcutaneous fat, indicated with the arrow. There are four left-sided smaller nodules. d) In diffusion weighted imaging (DWI) all of the nodules demonstrate restricted diffusion and enhancement.

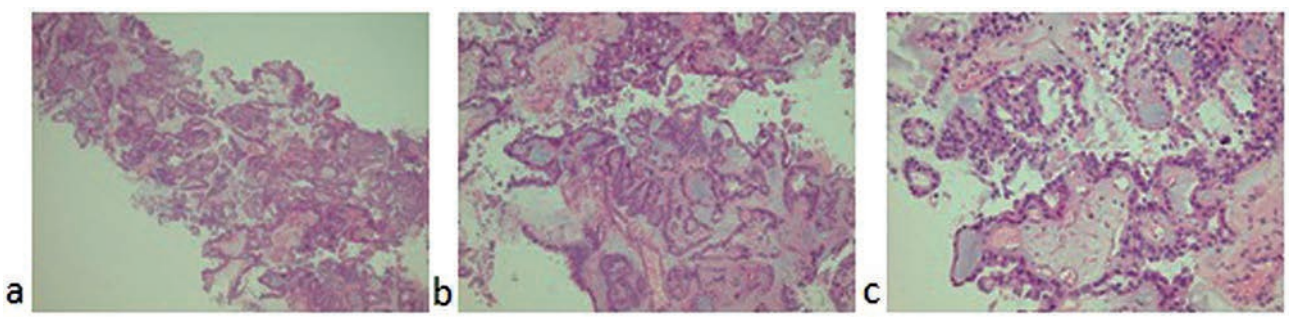

Figure 2. — Histopathology of subcutaneous nodule demonstrating features consistent with ovarian clear cell carcinoma, including Hobnailed appearance of tumour cells, cytoplasmic clearing, eosinophilic stromal cores in papillae, and papillary and glandular architecture. Haematoxiline and Eosin staining. Magnification a) ×5; b) ×10; c) ×20.

nodules (Figure 1), confirmed to be metastatic ovarian clear cell carcinoma on fine needle biopsy (Figure 2). A repeat PET scan demonstrated widespread metastatic disease in the liver and omentum. The Ca125 level was 193 U/ml. The patient was treated with two cycles of carboplatin and gemcitabine chemotherapy. A CT scan following these two cycles demonstrated progression of disease and the Ca125 level had risen to 2,400 U/ml; and therefore the chemotherapy regime was changed to liposomal doxorubicin. At the time of this report, the patient had received six cycles of chemotherapy with liposomal doxorubicin. The Ca125 had fallen to 300 U/ml and a CT scan demonstrated stable disease.

Discussion

Ovarian cancer is associated with the highest mortality of all gynaecological cancers [8]; and therefore gaining a greater understanding of the pathophysiology of ovarian

cancer is of upmost importance. Ovarian clear cell carcinomas, a subtype of epithelial ovarian cancers, typically present early in the disease process, however disease recurrence is common and often fatal [2]. Metastasis from ovarian cancer occurs predominantly by direct extension into the peritoneal cavity and dissemination via the peritoneal fluid [9]. Studies have found that approximately 30-38% of patients with epithelial ovarian cancer develop distant metastases [5-6]; and the presence of distant site metastases, including liver, lung, and pleural are associated with poor prognosis. As discussed in this paper, distant metastases to skin are very rare [5].

The pathophysiology contributing to skin metastases remains unclear. The majority of cases reporting skin metastases detail nodules in surgical sites and laparoscopic port sites, suggesting direct seeding may be an important factor [5, 10]. This case study details five subcutaneous nodules in the anterior abdominal wall, not associated with a surgical incision site. A recent case study has also presented the finding of subcutaneous nodules in the chest wall associated with metastasis from epithelial ovarian cancer [11]. This suggests that direct peritoneal spread may not be the only factor contributing to metastases to skin and likely lymphatic or haematogenous spread may be involved. Previous studies have shown that lymphatic invasion of ovarian tumours is associated with metastases to lymph nodes, small bowel, lung, and liver [12]. Further investigation is required to identify the underlying mechanism contributing to subcutaneous metastases from ovarian carcinoma.

Skin and other distant metastases occur at late stages and are associated with disseminated disease, as was the finding in this case. Management options at this late stage are unclear; however palliative approaches have been suggested to be appropriate [5, 13]. Surgical resection of the subcutaneous nodules has also been proposed to increase survival time [14]; although this may not be suitable in the setting of widespread disease [13].

Oncologists should be aware of subcutaneous nodules as a presentation of ovarian cancer recurrence and investigate any new development of subcutaneous nodules accordingly.

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Presentation of a patient with *in situ* amelanotic melanoma of the vulva

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Summary

Amelanotic malignant melanoma of the vulva is extremely rare. The authors describe here a case of amelanotic malignant melanoma of the vulva, occurring in a 71-year-old woman without any clinical symptoms. The woman had a small nodular lesion in the left labia majora. Local excision was performed. Histological examination revealed an *in situ* malignant melanoma without any evidence of invasive disease. All suspicious lesions in the vulva region, even if there are no clinical symptoms, should be biopsied, and if an *in-situ* melanoma is identified, partial or total vulvectomy should be considered.

Key words: Amelanotic melanoma in situ; Vulva; Treatment.

Introduction

Vulvar melanoma *in situ* is rare. Like oral mucosal melanomas, vulvar *in situ* lesions are considered precursors of invasive melanoma and the progression is often multifocal [1]. The majority of vulvovaginal melanomas present with relatively advanced tumors while *in situ* lesions alone are not usually recognized [2,3]. The vulvovaginal junction at the introitus is a high risk site for vaginal and vulvar melanoma. The intraoperative management requires accurate and complete assessment of lesions that extend both laterally and in depth in melanoma *in situ* [4]. It is not generally appreciated that melanoma of the vulva and vagina have more in common with mucosal melanomas such as those in mouth, which appear to be biologically different to cutaneous melanoma [5]. Approximately 3% of all melanomas diagnosed in women are located within the genital tract, predominantly affecting the vulva, followed by vaginal melanoma [6]. Vulvar melanoma represent less than 1% of all melanomas. Moreover it is the second most common vulvar malignancy, as it represents from 3.4% to 10% of cases among vulvar neoplasms [7]. Melanoma *in situ* in about 20-45% of cases does not present any clinical symptoms [8]. Amelanotic melanoma of the vulva is extremely rare; in a recent review, less than 10% were found to lack pigmentation [9]. In this paper, the authors present a rare case of amelanotic melanoma of the vulva, without any clinical symptoms recognized in random check up for uterine prolapse.

Case Report

A 71-year-old woman with a rectocele presented in 2005. In the last period she had various complaints: gradually increasing lower bowel pain and defecator dysfunction without any fecal incontinence. The following previous operations were reported: a) a total hysterectomy with bilateral adnexectomy, b) cholecystectomy, and c) once umbilical hernia operation, at 30, ten, and three years ago, respectively. The gynaecological and transvaginal examinations were unremarkable. Blood chemistry showed no abnormality. Accidentally a small nodular lesion was observed in the left labia majora. Macroscopically the features of the lesion were normal without any colour changes with a size to 0.4 cm. The physical examination detected a vulvar mass, 6×2×2 mm in size, without asymmetry irregular borders. No ulcers or bleeding was detected. Moreover no presenting clinical symptoms in vulva area were referred. For rectocele repair the woman underwent a posterior colporrhaphy and a vulva-biopsy. The ill-defined dermal lesion with extension into subcutaneous tissue was noted. The intra- and postoperative course was uneventful. The extracted material from left labia was submitted for a histopathological examination. Histological examination revealed an *in-situ* malignant melanoma without any evidence of invasive disease. The tumor was composed of large neoplastic cells with abnormal, hyperchromatic nuclei, having abundant eosinophilic, often clear, cytoplasm (Figure 1). The lesion lacked melanin, necrosis or haemorrhage. There were, however, focal areas of melanocytic dysplasia of various degrees. A scattered mononuclear cell infiltrate was noted in the superficial dermis. Immunohistochemically, tumour cells stained positively for HMB-45, Melan A, and S-100 protein. (Figure 2). The above described immunohistochemically findings revealed the diagnosis of primary amelanotic melanoma [10, 11]. The tumor was totally excised with adequate and histological tumor free margins. Seven days later after vulva biopsy, a magnetic resonance imaging did not show any metastases. Two weeks later

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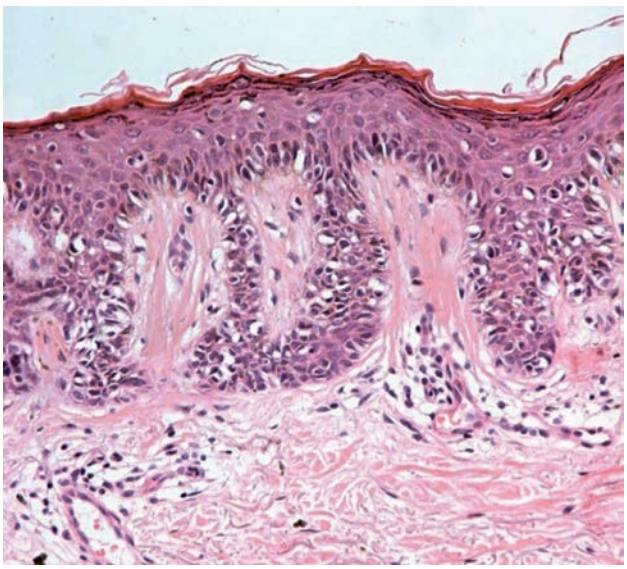

Figure 1. — Amelanotic vulvar melanoma in stu.

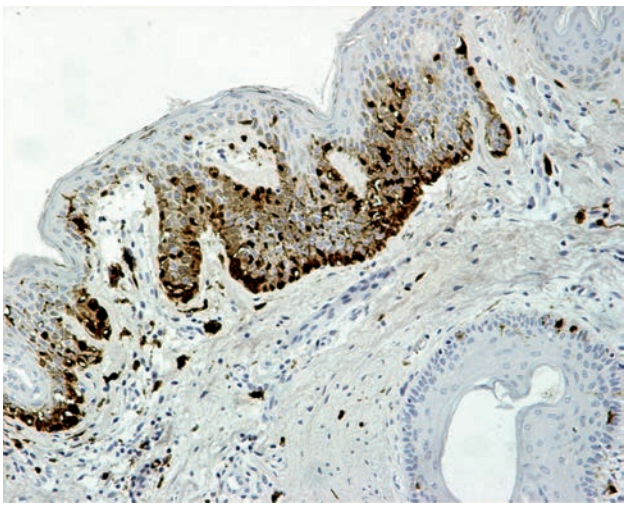

Figure 2. — Tumour cells stained positively HMB-45, Melan A, and S-100 protein.

Table 1. — *Invasive amelanotic melanomas of vulva*: *treatment modalities.*

Shetty K.J. *et al.,* 2012 [18]	23 years	Mass 5×6 cm	Inoperable/radiotherapy	Mass, odour, bleeding
Baderca F.L. *et al.*, 2008 [16]	78 years	Inner site right labium majus 5×2 cm	Hemi vulvectomy, resection of clitoris/polychemotherapy	Pruritus, swelling, bleeding
Oiso N. *et al.*, 2010 [14]	80 years	20 mm left labium majus	Excision of the tumor	Ulcerated nodule
An J. *et al.*, 2009 [10]	60 years	2 cm left labium minus	Radical vulvectomy and bilateral inguinal lymphadenectomy.	Ulcer, bleeding, burning discomfort
Ulmer A. *et al.*, 1996 [19]	60 years	Left labium	Hemi vulvectomy, resection of	Pruritus, bleeding
Hoffman U. *et al.*, 1995 [20]	55 years	1.3 cm right labium majus	clitoris, resection of the tumor	Nodular tumor

the patient underwent a partial vulvectomy. Histological examination of the surgical margins were again optimal tumor-free. The patient will have the first follow up examination six months post-operatively.

Discussion

Vulvar melanomas are usually pigmented or characterized by a painful or bleeding identified mass. The term amelanotic melanoma includes true amelanotic lesions with no pigmentation and melanomas with minimal residual pigmentation [12]. Most reported cases of amelanotic melanoma are metastatic, however they have been cases of amelanotic melanoma of primary lesion [13, 14]. The radiological diagnostic procedures which are used for confirmation of amelanotic melanoma, as computed tomography, magnetic resonance imaging, ultrasound, and proctoscopy are not specific to all cases of amelanotic melanoma, because approximately 30% them lack pigmentation. Only a histopathological examination can confirm an amelanotic melanoma. Histopathologically in primary lesions are found nested and single growth of atypical melanocytes in the surrounding mucosa and immunohistochemical markers as S-100, HMB-45, Melan A, and microphthalmia associated transcription factor. The primary modality of treatment is surgery [11, 15].

In this case a woman with a small nodular region in the left labia majora was presented. No pigmentation and no clinical symptoms as ulcers or bleeding were detected. The patient underwent a partial vulvectomy with tumor-free surgical margins. It is also important to mention that in the recent literature, only a few cases of amelanotic vulvar melanomas have been published and none of these have been found at such an early stage [10]. Furthermore other studies referred to ulcerated lesions of the vulvar region that was not observed in the present case. It is possible that the present authors found the lesion in a very early stage. In a recent study, only two cases of vulvar melanoma were diagnosed *in situ* and none was amelanotic [7]. A case of amelanotic melanoma of the vulva is referred by Baderca *et al.*, which was not *in situ* [16].

The treatment of the vulvar melanoma is local excision with tumor free margins. Many studies found no differ-

ences between radical rejection of the lesion with total vulvectomy and lymphadenectomy and more conservative techniques [17]. The present authors decided to perform a partial vulvectomy although the tumour was totally excised from the first time with adequate and histological free margins.

It is concluded that vulvar malignant melanoma is a rare and aggressive tumour. Standardized treatment of patients with malignant melanoma of the vulva is required for definitive treatment. Surgery should be performed in accordance with the accepted standards for cutaneous melanoma, however no treatment recommendations exist due to the rarity of this disease. The ABCDE scheme (asymmetry, border, irregularities, colour variation, diameter > five mm. enlargement or evaluation of colour change, shape, or symptoms) is very useful for early melanoma recognition [10]. All suspicious pigmented or unusually thick lesions in the vulva region should be biopsied for histopathological evaluation. The depth of these lesions is critical. Table 1 presents cases of invasive amelanotic melanoma and treatment modalities [18-20]. However current literature research has not referred any other case of amelanotic melanoma vulva *in situ*.

The gynaecologist should take into consideration that lesions of the vulva could be suspicious for melanoma even when there is no melanin pigmentation and no clinical symptoms. Amelanotic melanoma may present in a great variety of clinical features and should be considered in the different diagnosis of any nonpigmented lesion located in uncommon position of the vulva. Local excision and partial vulvectomy is recommended for patients with malignant melanoma *in situ* of the vulva. Therefore, the importance of self-examination and early diagnosis of melanoma of the vulva should be emphasized, and accurate inspection of the vulva should be indispensable during routine gynaecological examination. Any lesion in vulva should be considered likely to be a melanoma. Standardized documentation of clinical and histopathological parameters is needed for grouping of cases for future comparative studies.

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Gynandroblastoma of postmenopausal women: a case report

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Summary

Gynandroblastoma, an extremely rare ovarian tumour that usually consists of both Sertoli stromal cell and granulosa cell tumours, often produces both androgenic and estrogenic effects. The authors herein report a case of gynandroblastoma with the longest disease-free period reported to date. A 66-year-old woman without metrorrhagia or hirsutism presented with abdominal pain and slightly elevated serum estradiol levels. Her uterus was enlarged, and endometrial curettage performed to reduce endometrial thickness prior to laparotomy led to a diagnosis of atypical endometrial hyperplasia. She was diagnosed of ovarian tumour. The pathology report revealed that the right ovarian tumour was a "gynandroblastoma". Such lesions are classified as borderline malignant. Postoperative adjuvant therapy was not administered in this case because only a few recurrent or fatal cases have been reported. The lesion was classified as pT1aN0M0 according to Union for International Cancer Control (UICC). The patient is alive and has been disease-free for 77 months post-surgery.

Key words: Gynandroblastoma; Sex cord; Stromal cell tumours; Granulosa cell tumor; Sertoli cell tumour.

Introduction

Gynandroblastoma is an extremely rare tumour consisting of female-type sex-cord stromal tumour cells and male-type sex-cord stromal tumour cells, and each of these components accounts for at least 10% or more of the mass. These lesions usually present with adult-type granulosa cells and Sertoli cells in young adults [1] and often induce signs of masculinization and feminization due to the effects of tumour-producing sex hormones, such as estradiol and testosterone.

Although convalescence after tumour excision is thought to be associated with a good prognosis, gynandroblastoma is usually borderline malignant. In addition, there are currently no large-scale studies available regarding the prognosis of this disease, and late-onset recurrence and subsequent death may occur in some cases [2].

The authors herein report a case of gynandroblastoma involving the longest disease-free period.

Case Report

A 66-year-old woman with mild lower abdominal pain for a month presented to this hospital. Her gynaecological history was unremarkable (G5P3, menopause at age 48). She had asthma and had undergone cholecystectomy at age 48. She did not smoke or consume alcohol. Her general condition was stable (consciousness, clear; pulse rate, 78 per minute; body temperature, 36.8°C; body mass index, 27.8 kg/m^2) except for a mildly high blood pressure at 190/80 mmHg. She did not present evidence of feminization or masculinization.

On physical examination, an elastic-hard mass, the size of a newborn's head (11 cm), was palpable with tenderness in the middle of the lower abdomen. The uterus was enlarged for her age at 10×6 cm, the endometrium was thickened at two cm, and a solid-cystic mass measuring ten cm in diameter presented as a right adnexal lesion on transvaginal ultrasound. Magnetic resonance imaging (Figure 1) showed that the solid part had a relatively high intensity, whereas the cystic part showed a mixture of low-and high-intensity features on a T2W1 image. Additionally, a nutrient vessel of the tumour from the uterus was suspected. Thoraco-abdominal computed tomography revealed no apparent metastasis or enlarged lymph nodes. The complete blood count and biochemistry tests results that the parameters were within the normal range, although CA-125 (52.2 U/ml) and serum estradiol (146 pg/ml) levels were slightly elevated. Endometrial curettage, performed for endometrial thickness prior to laparotomy, led to a diagnosis of atypical endometrial hyperplasia. She underwent surgery after the diagnosis of right ovarian tumour, which was suspected to be a granulose cell tumour.

Accumulated yellowish serous ascites (600 ml) were submitted for cytological analysis, and the result was negative. The uterus, left adnexa, and intestines were adhesion-free, but the enlarged right ovary, the size of a new-born's head, strongly adhered to the retroperitoneum. Because of the ascites and adhesion, a malignant tumour was suspected. Therefore, simple hysterectomy and bilateral adnexectomy were performed. The authors did not pathologically diagnose the tumour during surgery.

The right ovarian tumour had an 11-cm diameter and 450-gram weight. It was elastic-hard with a mostly smooth surface, although papillary structure was observed in some areas. Macroscopic examination of the excised surface of the tumour revealed a yellowish solid component with bleeding, necrosis, and various sized cysts containing serous liquid. There were some protrusions on the cyst wall. Endometrial thickness was apparent, but no noticeable myometrial proliferation was observed (Figure 2).

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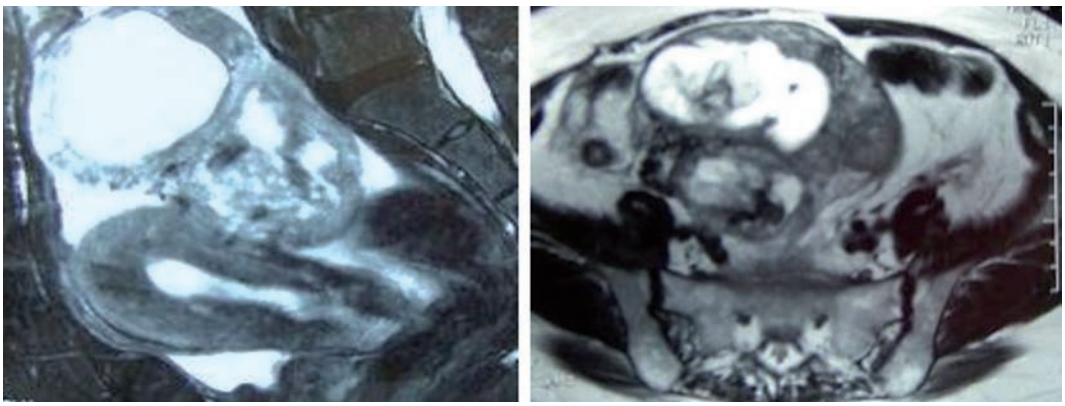

Figure 1. — Magnetic resonance imaging showing a solid component with a relatively high intensity, whereas the cystic part shows a mixture of low-and high-intensity features on a T2W1 image

Histologically, the tumour comprised of two different elements (Figures 3 and 4): "granulosa cell tumour," which shows characteristic features such as Call-Exner bodies (Figure 3c), nuclei with folds and grooves (Figure 3d), microfollicular pattern (Figure 3e), trabecular architecture, and insular formation, and "Sertoli cell tumour" with its typical tubular formation, such as hollow tubules (Figure 3f) and immature tubules (Figure 3g). Eighty percent of the tumour was granulosa cell tumour and the remaining 20% was Sertoli cell tumour (Figure 4). Tumour cells were immunoreactive for inhibin, cytokeratin AE1/AE3, progesterone receptor, and calretinin in both granulosa and Sertoli cell tumour elements. Based on these findings, the tumour was diagnosed as a gynandroblastoma. Thickened endometrium histologically showed simple endometrial hyperplasia, and no remarkable findings were observed in the left ovary.

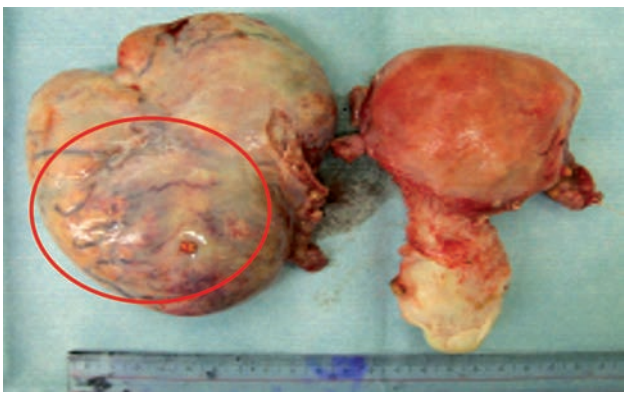

Figure 2. — Macroscopic findings of the excised specimen.

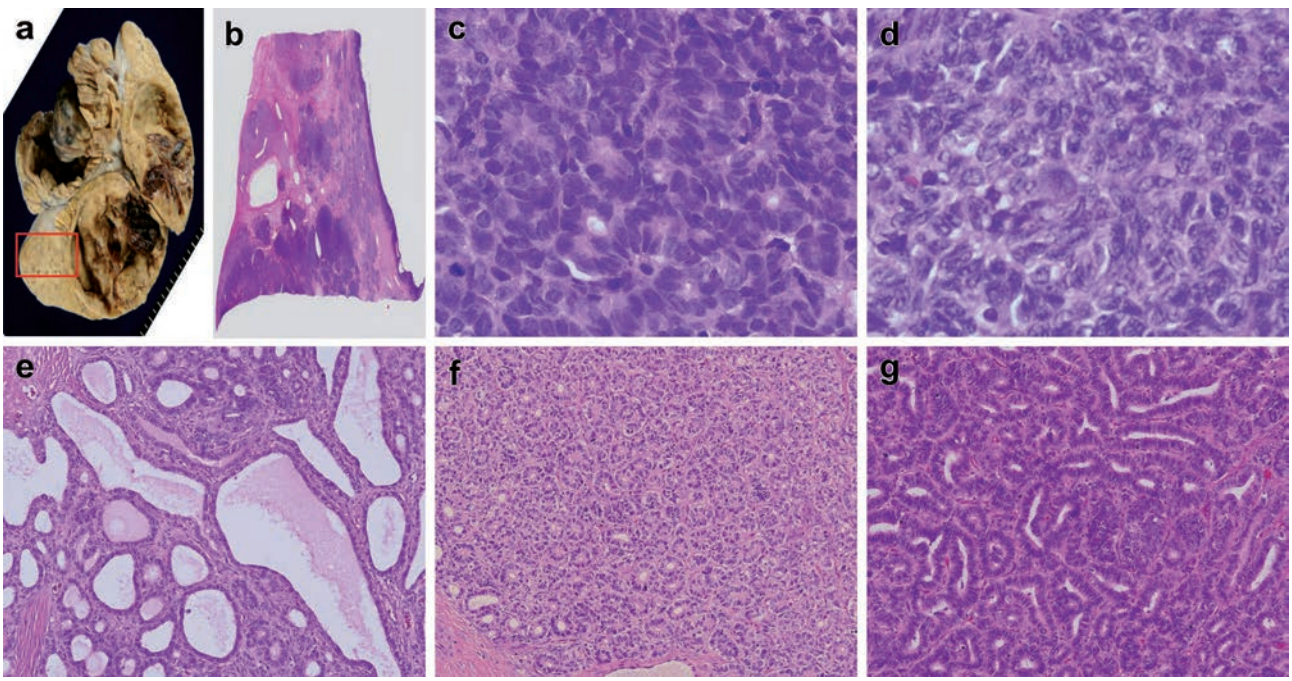

Figure 3. — Pathological findings. (a) Macroscopic findings of the excised surface of the tumour after formalin fixation. (b) Haematoxylin and Eosin staining of the portion of the tumour seen in the red frame in (a). (c) Call-Exner bodies of the granulosa cell tumour. (d) Nuclear groove of tumour cells of the granulosa cell tumour. (e) Microfollicular pattern of the granulosa cell tumour. (f) Hollow tubules of the Sertoli cell tumour. (g) Immature tubules of the Sertoli cell tumour.

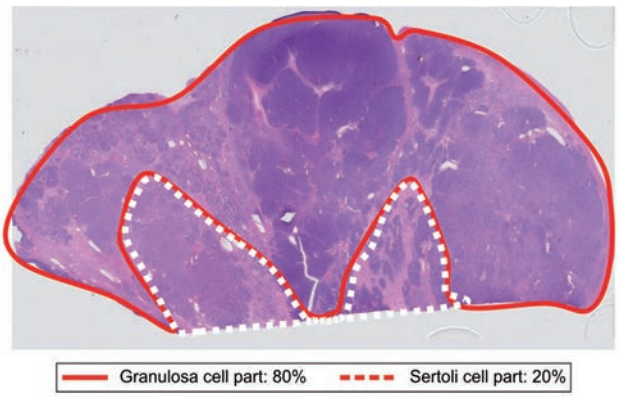

Figure 4. — Distribution of granulosa cell tumour and Sertoli cell tumour in the histologic section.

After surgery, the patient and her family were provided information on gynandroblastoma. Although the patient was informed of the possible benefits of adjunctive therapy that the treatment of choice for borderline tumours, the patient decided to undergo follow-up without adjunctive treatment. Written informed consent was obtained from the patient. She has been healthy without any relapse of the tumour during the 77 months of follow-up post-surgery.

Discussion

Embryologically, Sertoli cells and Leydig cells are thought to develop from primitive embryonic marrow, and granulosa cells develop only from cortex sex cords [3, 4]. The exact origin of gynandroblastomas is unknown. Some reports state that gynandroblastoma is a simultaneous tumorigenic transformation of marrow and cortex. In contrast, it has also been reported that gynandroblastomas develop from undifferentiated gonadal mesenchymal cells that have the potential to differentiate into both sexes.

Macroscopically, gynandroblastoma is a solid tumour with cystic formation, and the colour of the division surface is white or flavedo. The size of the tumour ranges from two to 20 cm [5]. When masculinization and feminization exist simultaneously, gynandroblastoma can be clinically suspected; however, the diagnosis of gynandroblastoma using only the symptoms is sometimes difficult because of the opposing effects of each hormone. The differential diagnoses include granulosa cell tumours, Sertoli-stromal cell tumours, small cell carcinoma, endometrial stromal sarcoma, and endometrioid adenocarcinoma.

In the present case, the patient did not have metrorrhagia or signs of masculinization, and her serum estradiol levels were slightly elevated. Her uterus was enlarged for her age. The major complaint of abdominal pain led to the diagnosis of ovarian tumour, and granulosa cell tumour was suspected because of the presence of atypical endometrial hyperplasia resulting from the high serum estradiol levels.

Since there are not many long-term follow-up studies, the present authors suggest careful observation of patients with such tumours. The patient received treatment and underwent long-term follow-up at this hospital; however, more such cases are required to accumulate evidence.

Conclusion

Granulosa and Sertoli cell tumours are categorised as being borderline malignant. The prognosis of both tumours is generally excellent, although a few lesions display malignant biologic behaviour. The current patient has remained healthy without any relapse of the tumour for 77 months of follow-up after surgery. Since gynandroblastoma is a rare tumour, careful follow-up is important in order to determine the biologic behaviour of such lesions.

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Partial regression of a hydatiform mole with coexisting live fetus in a twin gestation: case report

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Summary

Pregnancies resulting in viable fetuses are extremely rare in accompanying a hydatiform mole, often due to the development of maternal complications, including preeclampsia and vaginal bleeding. The risk for gestational trophoblastic neoplasm is another concern because of the delayed evacuation of the molar tissue. In this paper, the authors present a case of complete mole hydatiform with a live co-twin fetus (CHMLF) resulting in the delivery of a healthy male infant with the partial regression of the molar tissue and the decline of serum beta human chorionic gonadotropin (β-hCG) during the pregnancy. In the management of CHMLF, each patient must be considered individually and eligible patients can be followed in the absence of serious maternal complications. Serial ultrasound examinations and close clinical and laboratory surveillance of the mother are certainly indicated.

Key words: Complete mole hydatiform; Twin pregnancy; Gestational trophoblastic neoplasm.

Introduction

Pregnancies consisting of a live fetus accompanying a hydatiform mole are uncommon and comprise one in 22,000–100,000 pregnancies [1]. Among them, the pregnancies resulting in viable fetuses are extremely rare, often due to the development of maternal complications, including preeclampsia and vaginal bleeding. The risk for gestational trophoblastic neoplasm is another concern because of the delayed evacuation of the molar tissue. [2]

In this paper, the authors present a case of complete mole hydatiform (CHM) with a live co-twin fetus (CHMLF) resulting in the delivery of a healthy male infant with the partial regression of the molar tissue and the decline of serum beta human chorionic gonadotropin (β-hCG) during the pregnancy.

Case Report

A 25-year-old woman, gravida 2, para 1, at 22 weeks' gestation was referred to the present institution on suspicion of CHM and CHMLF. Her previous medical history was unremarkable and her obstetric history included one term vaginal birth. She had no problems with the pregnancy except for slight vaginal bleeding and mild hyperemesis by that time. Ultrasound examination revealed a live fetus with normal placenta and adjacent to the placenta a well-defined multi-cystic snowstorm-like mass which was compatible with CMH. This cystic area measured 12.5×7 cm (Figure 1). The amount of amniotic fluid was normal and there were no signs of growth retardation or fetal anomalies. The serum level of β-hCG was > 10,000 IU/ml. CHMLF was strongly suspected. The risk of possible maternal complications, fetal malformations, and subsequent malign transformation were explained and the couple was counseled for termination, but they chose to continue this pregnancy and declined any invasive prenatal testing to confirm the karyotype of the fetus. During the expectant management there were no major maternal complications (thyrotoxiosis, preeclampsia, anemia). Serial ultrasound examinations performed at two-week intervals, demonstrated normal fetal growth and a reduction in size of the molar tissue. Recurrent vaginal spotting continued in the second and third trimester. On the last ultrasound, molar tissue was observed thoroughly small on one side of the uterus. At 36 weeks, a cesarean section was performed because of vaginal bleeding, and a 2,630-gram male infant with an Apgar score of 9 and 10 at one and five minutes was delivered. The placenta and grape-like mass were removed manually. The pathologic examination of the placenta revealed 6.5×4 cm focal area which was compatible with CMH (Figure 2).

Immunohistochemical stains with the P57KIP2 anti-body were helpful in the differential diagnosis of complete hydatiform moles. Expression of P57KİP2, a paternally imprinted gene, is either absent or low in trophoblast in cases of complete moles in contrast to diffuse staining in partial moles and non-molar placentas. In present case, the villous stromal cells and cytotrophoblastic cells did not show P57KIP2 immunoreactivity, while the intermediate trophoblastic cells were positive (Figure 3) [3]. The karyotype in cultured cells from the molar area was diploid. The serum β-hCG level was 3,912 IU/ml at the delivery and patients followed by weekly serum β-hCG measurement. β-hCG levels decreased gradually. At second month of postpartum period the patient's β-hCG persisted at the levels of 56 and 60 IU/ml. Metotrexate with leucovorin rescue treatment was started and β-hCG level returned to normal after third course. No evidence of persistent gestational trophoblastic disease (PTD) was found at first year of postpartum period.

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Figure 1. — Ultrasound photograph at 22 weeks' gestation showing complete hydatiform mole (upper part) and normal placenta with a fetus. The molar tissue was 12.7×7 cm.

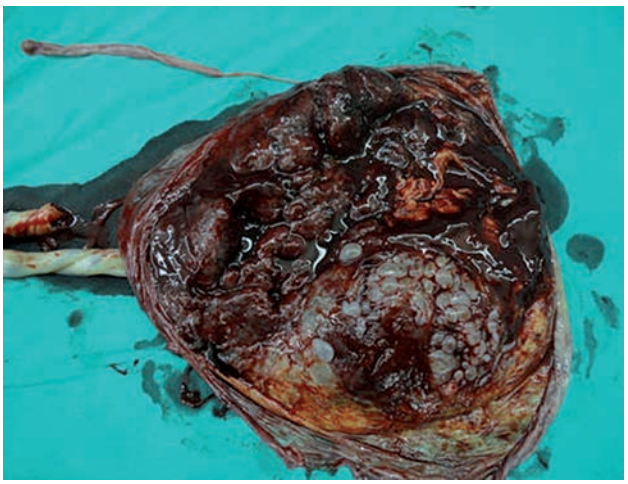

Figure 2. — Macroscopic photograph of the placenta (15×17 cm) and the molar tissue 6.5×4 cm.

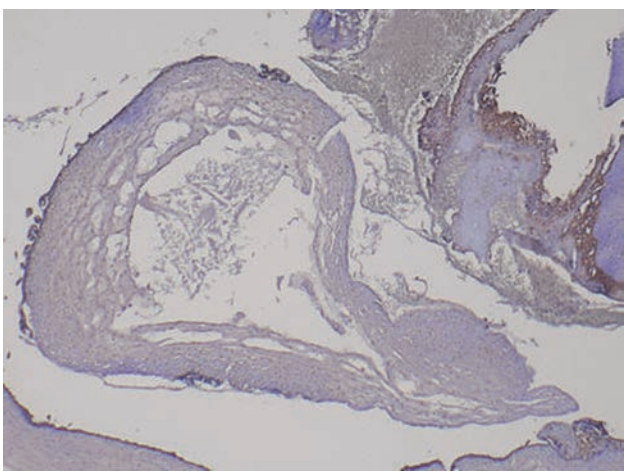

Figure 3. — p57KIP2 immunostaining of a complete hydatiform mole. The villous stromal cells and cytotrophoblastic cells do not show p57KIP2 immunoreactivity, while the intermediate trophoblastic cells were positive for p57.

Discussion

The CHMLF is a relatively uncommon event with a quoted range of incidence from one in 10,000 to one in 100,000 pregnancies [4]. Most of these cases either aborted or resulted in stillbirth and only a few with a living newborn. There are, overall, about 200 cases of twin pregnancy with CHMF documented to date in the literature and only 30 cases of twin pregnancy with CHMF resulting in a live birth documented in detail in the literature. [5]

Clinicians and parents with CHMLF cases encounter a clinical dilemma, as they have to decide between continuation or immediate termination of the pregnancy. The problems in the management of CHMLF involve the risks of fetal abnormality, malignant trophoblastic change, and severe maternal complications [6, 7]. The rate of pregnancy termination due to maternal complications is different in various reports. Fishman *et al.* [8] reported their termination rate as 71% because of maternal complications. This high rate may be due to spontaneous abortions and intrauterine deaths in their series. Conversely Sebire *et al.* [9] reported that only 4% of the pregnancies were terminated because of maternal complications.

As with the singleton molar pregnancies, some clinical criteria of aggressivity for the mother, such as maternal age, parity, uterine size, serum level of β-hCG before evacuation, signs of preeclampsia, and the existence of theca lutein cysts have been studied, but none of them predict progression to PTD [10]. Bristow *et al.* compared the clinical features of CHM in which the fetus remained viable and the pregnancies terminated or ended in stillbirth. In the non-viable group, the peak serum level of β-hCG was higher, and uterine height was greater than expected. In viable molar cases, the trophoblast was less aggressive or progressed to spontaneous degeneration. Spontaneous intrauterine fetal death appears to be very common in CHM with a live co-twin, mostly before 24 weeks [11].

The risk for PTD in the CHMLF cases is not higher than the risk in single molar pregnancies. Bristow *et al.* [11] reported that PTD occurs in 68.4% of CHMLF gestations delivered before fetal viability and 28.6% of those resulting in a living fetus. They concluded that the advanced gestational age required to produce a viable, surviving fetus is not an independent risk factor for the development of PTD. Several studies have shown that the risk level does not change with advanced gestational age [12, 13].

Marcorelles *et al.* [14] suggested that there are two types of spontaneous evolutions during the second trimester of pregnancy: either the molar part becomes quiescent, allow-

ing the pregnancy to continue, or it continues to grow extensively, leading to fetal death and maternal complications.

In the present case during the follow up, no maternal and fetal complications were observed and the pregnancy ended with the delivery of a live baby. The molar tissue regressed and the size of it decreased significantly during the pregnancy (partial regression). After delivery, patients were followed by serial β-hCG measurement; when the β-hCG levels persisted patients were diagnosed as gestational trophoblastic neoplasia (GTN). Because the patients were evaluated as low risk GTN, single-agent methotrexate treatment was administered. Negative β-hCG level was achieved and no recurrence was detected during a one-year period. In the present authors' opinion, in the management of CHMLF each patient must be considered individually and eligible patients can be followed in the absence of serious maternal complications. Serial ultrasound examinations and close clinical and laboratory surveillance of the mother are certainly indicated.

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EJGO European Journal of Gynaecological Oncology

Misdiagnosed ovarian Krukenberg tumor during pregnancy with virilization

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Summary

Krukenberg tumor with pregnancy is rare but it is a challenge for treatment and diagnosis. The authors report a case of a 29-week pregnant patient with a massive bilateral Krukenberg tumor which was misdiagnosed as myoma preoperatively and as ovarian stromal tumor intraoperatively. Prenatally the woman was asymptomatic except for preeclamptic symptoms, but red acne on the skin and elevated testosterone were observed. Pelvic ultrasound detected a heterogeneous solid mass mimicking a subserous myoma. The deterioration of preeclampsia prompted a cesarean section, but the neonate died nine days after he was born. A bilateral adnexal mass was found and considered as stromal tumor by frozen section because of luteinization of the stroma. The final pathology showed low differentiation adenocarcinoma of ovary, which was confirmed by gastric biopsies. The patient had undergone chemotherapy 16 times without surgical debulking and she was in generally well 1.5-year follow-up.

Key words: Ovary; Krukenberg tumor; Pregnancy; Virilization; Subserous myoma.

Introduction

The presence of adnexal masses during pregnancy ranges from 1:81 to 1:2,500 pregnancies, but only 3% of these masses are malignant [1]. A Krukenberg tumor is an ovarian adenocarcinoma metastasis from a primary malignancy of the gastrointestinal tract, with 76% originating from the stomach, and it accounts for 1~2% of all ovarian tumors [2]. The incidence of gastric cancer in pregnant women was only 0.016% even in Japan where a high rate of gastric cancers was reported [3]; hence Krukenberg tumor during pregnancy is even rarer.

The patient with Krukenberg tumor can be pregnant because the ovary still has a part of the normal cortex and hence does not affect ovulation. A Krukenberg tumor with pregnancy is so uncommon and due to the lack of treatment guidelines, it is easily misdiagnosed, thus presenting a challenge for the physician. The authors present a case report of a woman 29-weeks pregnant patient with massive bilateral Krukenberg tumors, which was misdiagnosed as subserous myoma preoperatively and ovarian stromal tumor intraoperatively. The aim was to provide clinicians with the clinical manifestations of this rare case and provide some information about better clinical diagnosis and treatment of the disease.

Case Report

A 26-year-old nulliparous woman with preeclampsia was referred to the Obstetrics Department at 29^{+2} weeks of gestation, with complaints of intermittent headache and dizziness over the past three weeks and clouded vision for one day. She complied with her antenatal schedule well and generally had no abnormalities detected during the course of pregnancy until recently. At eight weeks of gestation, progesterone treatment was once given because of vaginal bleeding. Small red acne on the skin of the neck and the chest which was androgenized body feature was observed since she was pregnant. She denied any past medical problems and was not taking any medications prior to her pregnancy. Additionally, she denied the use -of tobacco, alcohol, or illicit drugs. The family history was noncontributory.

The physical exam found her blood pressure to be 160/120 mmHg and it indicated a gravid uterus at around 28 weeks of gestation. Lower limbs had slight edema. A gynecological examination revealed no clitoral enlargement. Laboratory findings showed normal electrolytes. Hemoglobin was 135 g/L and platelets were 379×10^9/L. Urine analysis revealed no evidence of leucocytes and blood. However, the quantitative 24-hour urine collection for protein was 0.78 grams (normal level during pregnancy is 0-0.3 grams/24 hours). Carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9) levels were normal, while the cancer antigen 125 (CA 125) was found to be elevated at 92.49 U/ml (normal range: 0-39 U/ml) and alphafetoprotein (AFP) was 79.19 ng/ml (normal range: 0-20 ng/ml). Circulating levels of testosterone were elevated to 10.91 ng/ml (normal range: 0.06-0.82 ng/ml), while other hormones such as estradiol and aldosterone were in the normal range. 17-OH-progesterone was 4.96 μg/L (normal range: 0.40-1.02 μg/L).

An ultrasound revealed a normal fetal intrauterine pregnancy but detected a heterogeneous solid mass mimicking a subserous myoma originated from uterus, with regular echogenic margin and a texture, 11.3×9.4×6.8 cm in size, just the back of the cervix, without ascites (Figure 1A). The blood flow signals were seen in the mass. The image was so similar to that of a subserosal myoma that the authors did not perform further imaging studies such as CT or MRI.

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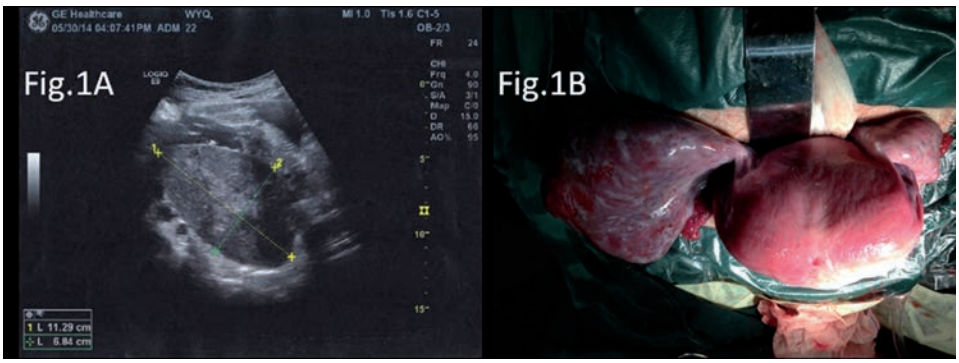

Figure 1 — A) Ultrasound showing a heterogeneous solid mass mimicking a subserous myoma, measuring 11.3×9.4×6.8 cm. B) During cesarean section, presence of a bilateral adnexal masses measures 12 cm in its greatest diameter in the right ovary, and 5 cm in the left.

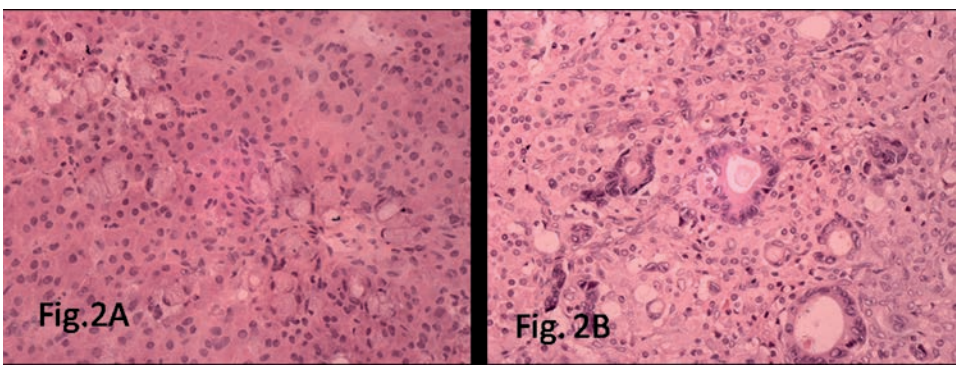

Figure 2 — A) Intraoperative frozen section of the ovary biopsy showing small groups of signet-ring cells scattered in the stroma, which is remarkably luteinized and misdiagnosed as a sclerosis stromal tumor (H&E, ×400). B) Paraffin section of the ovary (final pathology) showing the tumor tissue composed of numerous mucin-filled signet-ring cells and several intestinal-type glands. (H&E, ×400).

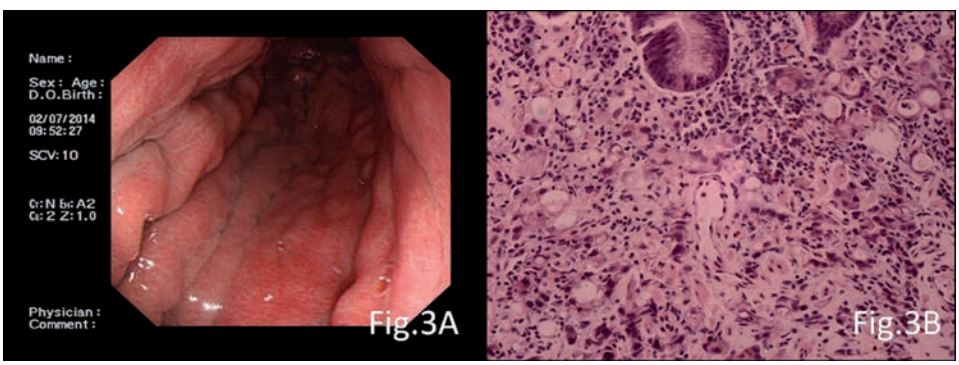

Figure 3 — A) Esophagogastroduodenoscopy showing mucosal fold thickening on the non-peristaltic stomach wall along the greater curvature. B) Gastric biopsy shows a poorly differentiated adenocarcinoma in the lamina propria of stomach. The tumor is characterized by signet-ring cells, tubular glands, and solid tubules (H&E, ×400).

The patient with preeclampsia was stabilized with magnesium sulfate and anti-hypertensive therapy, monitoring for maternal and fetal well-being. One week later, the patient experienced abdominal distension and blood pressure increased to 180/128 mm/Hg, and ultrasound showed ascites about 2,000 ml and lactate dehydrogenase (LDH) was up to 407 U/L (normal range: 40-250 U/L). Urine analysis revealed quantitative 24-hour urine collection for protein to be 12.56 grams.The deterioration of preeclampsia prompted the decision of termination of pregnancy.

Cesarean section was conducted under spinal anesthesia and one 1,100-gram male fetus was born with Apgar scores 9 and 9 at one and five minutes and was then admitted to the neonatal intensive care unit. There were no neonatal apparent anomalies but the neonate developed respiratory distress syndrome that was treated with surfactant. However, his situation was getting worse and at last family gave up the treatment, and nine days after he was born, the newborn died. After the closure of the uterus during operation, the patient was found to have a bilateral adnexal mass measuring 12 cm in its greatest diameter of the right ovary and 5 cm for the left (Figure 1B). The right ovary biopsy was processed for frozen section investigation, which was considered sclerosis stromal tumor (Figure 2A). Frozen section for the left ovary biopsy was also subsequently considered a stromal tumor. Palpation of the upper abdomen showed macroscopically normal liver, spleen, peritoneum, omentum, small and large intestines, but the greater curvature of the stomach was found to be a bit stiff, which suggested a primary gastric cancer. Although the gynecologist doubted a Krukenberg tumor during exploration, intraoperative pathological examination indicated there was no lesion for malignancy. Therefore the authors decided neither to remove the ovaries and nor to enlarge the operation, and the next suggested therapy was to be given after the final pathologic result and gastric examination.

The final pathology showed low differentiation adenocarcinoma of bilateral ovary, which was confirmed with numerous mucin-filled signet-ring cells and several intestinal-type glands, and indicated a possible metastasis from a gastric origin (Figure 2B). This was further supported by the immunochemistry results. The tumor was im-

munoreactive to CK7 and CK20, which suggested a gastro- pancreatobiliary origin. Based on the intraoperative exams, an esophagogastroduodenoscopy was performed, which revealed patchy areas of erythema and mucosal fold thickening on the non-peristaltic stomach wall along the greater curvature (Figure 3A). Multiple biopsies were obtained that showed the presence of a poorly differentiated adenocarcinoma in the lamina propria of stomach (Figure 3B).

Ten postoperative days later, the patient was discharged from the hospital after recovery of the preeclampsia. Since she was diagnosed as having Stage IV gastric cancer, palliative chemotherapy was scheduled. Then the patient had undergone chemotherapy 16 cycles without surgical debulking and she was generally well when the authors had performed a 1.5-year follow-up.

Discussion

Generally, a Krukenberg tumor is usually considered as an advanced presentation of gastric cancer, although less than one-third of the primary sites were appendix, colon, breast, small intestine, rectum, gallbladder, and urinary bladder [4]. The special tumor has different clinical manifestations and the patients usually present with symptoms related to ovarian involvement such as abdominal pain and distention [4]. However, the remainders of patients present with non-specific gastrointestinal complaints or are asymptomatic. Kiyokawa *et al.* performed an analysis of 120 Krukenberg tumors and found that the dominated clinical presentation was usually abdominal swelling or pain, while 17 patients had abnormal vaginal bleeding. Ascites was present in 43% of the cases. Sixty-three percent of the tumors were documented to be bilateral [5]. However, during pregnancy the diagnosis of Krukenberg tumor poses a challenge because of its extremely rare incidence [1, 6-10]. Moreover, gastric cancer often presents with some symptoms such as nausea and vomiting, which are common experiences during pregnancy, affecting most of pregnant women. The abdominal pain and distention can be explained by pregnancy, ovarian masses, and ascites. So pregnancy easily masks the symptoms of recurrence and delays the diagnosis and treatment. In this case, the main presentation was typical preeclampsia symptoms such as high blood pressure, edema, proteinuria, and dizziness. Another important reason why the authors did not diagnose gastric cancer earlier was because she had no known risk factors for gastrointrstinal malignancies. She was just a 26-year-old and had no known H. pylori infection and non-smoker status. Furthermore, she was largely asymptomatic of features suggesting upper gastrointestinal malignancy.

Ultrasonography and MRI are essential in the workup for imaging of adnexal masses during pregnancy. Certain sonographic findings can indicate a Krukenberg tumor. Usually abdominal and pelvic ultrasonography and MRI reveal bilateral and solid ovarian masses, but cystic masses can also occur. In pregnancy, the echo structure of Krukenberg tumor was homogeneously hyperechoic and abundant vascularization and a main vessel with a tree-shaped structure on the color Doppler examination was observed [11]. However, it may be confused with other adnexal masses, such as teratomas and corpus luteum cysts, which are more common during pregnancy. At times, ovarian neoplasm should be identified with a subserosal uterine myoma with pedicle [12], because the sonographic texture of the myoma has a solid mass and regular echogenic margin. In the present case, despite undergoing multiple ultrasound scans, no pelvic abnormalities and adnexal masses had been detected until the third trimester of pregnancy. Perhaps the reason was the mask of enlargement of the uterus with pregnancy. Unfortunately, the ovarian tumor was misdiagnosed as subserous myoma, which allowed clinicians to ignore the further examination of the mass.

To predict ovarian cancer during pregnancy, the role of tumor markers remains controversial. Firstly, it is infrequent that pregnancy associated pelvic masses are malignant. Secondly, with gestational age the interpretation of these tumor markers should vary. Several of the tumor markers used to diagnose ovarian cancers are difficult to interpret during pregnancy, because oncofetal antigens are often involved in biological functions associated with fetal development, differentiation, and maturation [13]. So the authors thought mild increase of CA 125 and AFP in this case had low sensitivity and specificity for ovarian tumor. Interestingly, the patient presented some signs of virilization after she was pregnant. Those virilizing symptoms during pregnancy could be easily attributed to an androgen secreting pregnancy luteoma or endocrine diseases such as adrenal hyperplasia. However, many cases of virilization and hirsutism of mother in association with Krukenberg tumors during pregnancy have been reported in literature [5, 7, 9, 14], although it is still unclear why some Krukenberg ovarian tumors lead to androgen overproduction as presented in this case. The present patient was a rare case, but the authors did not recognize that the sign of virilization was presentation of ovarian metastatic tumor. Therefore, it should be emphasized that an important manifestation of the Krukenberg tumor with pregnancy is virilization by the hormone production, although only a small percentage of patients have endocrine manifestations.

Krukenberg tumors are sometimes misdiagnosed as Sertoli cell or Sertoli-Leydig cell tumors by the submitting pathologist, particularly when a prominent tubular component and luteinization of the stroma, sometimes accompanied by virilization, are encountered [5]. In this case, the tumor was regarded as ovarian stromal neoplasm through rapid frozen pathologic examination. The reason of misdiagnosis was due to a large number of luteinized stromal cells which could secret hormones including testosterone in the ovary (Figure 2A). Histologically, Krukenberg tumor often consist of mucus-producing glandular structures, small solid nests, and numerous signet-ring cells surrounded by partly luteinized ovarian stroma [15]. However, Sertoli and Sertoli-Leydig cell tumors are rarely bilateral, and although large empty rounded lipid vacuoles may be present, particularly in Sertoli cells, signet-ring cells containing mucin are not encountered [5]. The present patient with virilization revealed a stromal luteinization, and androgenizing hormone production re-

sulted from development of luteinized stroma in Krukenberg tumors. Elevated serum levels of hCG are thought to be the cause of the stromal luteinization of tumors during pregnancy, and the hCG dependence of virilizing Krukenberg tumors during pregnancy has been documented by both in vitro and in vivo hormonal studies [5].

An important factor that affects fetal survival is metastatic potential of the cancer of fetus [16]. Placental metastasis is a high risk factor for fetal metastasis. In order to avoid neonatal adverse outcomes, pathologic examination of the placenta is needed and the newborns should be followed up closely once placental metastasis is found [17]. In the present case, the placental tissue was not made a pathologic examination, so the authors still do not know whether the neonatal death was due to fetal metastasis.

Because of the rarity of Krukenberg tumor with pregnancy, there is insufficient information in the literature regarding guidance for appropriate treatment and management. Multidisciplinary team approach is absolutely the way to managing such a rare and difficult disease, including preoperative and intraoperative diagnosis and treatment. It was suggested that following a diagnosis of malignancy in pregnancy, the patient should be promptly transferred to a regional cancer facility that was equipped with a linked obstetrics and neonatal unit [6]. In spite of this, the overall prognosis of Krukenberg tumor is often poor if the primary tumor is found after metastasis to the ovaries, and even worse, if the primary tumor remains undetected [4, 8]. Jaspers *et al.* reported that the three-year survival rate was 8%, although this had less of an impact on fetal survival [18]. Outside of pregnancy, Krukenberg tumors had a median survival of 14 months [4]. However, possible early detection with debulking surgery, and aggressive targeted chemotherapy might improve the survival of these patients [19]. Fortunately, the patient in the present case was still alive after 16 months and she was treated with more than 16 cycles of chemotherapy.

Conclusion

Krukenberg tumor is rare during pregnancy and the patient may present with non-specific gastrointestinal complaints or are asymptomatic. Virilization by the hormone production perhaps is an important manifestation of the tumor with pregnancy. The physicians should be suspicious of the presence of such tumor in young pregnant women with enlarged ovaries and virilization.

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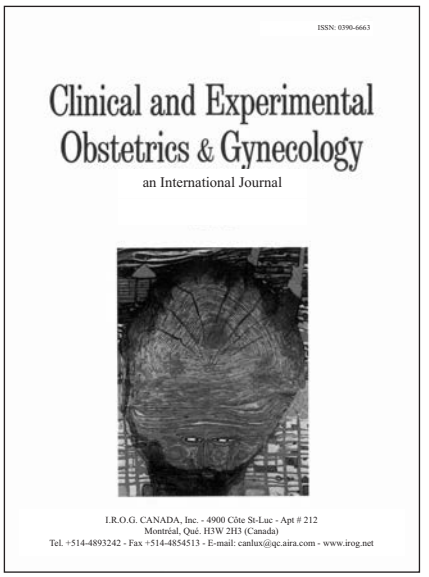

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